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THE UNIVERSITY OF ALBERTA

PHYSICAL, CHEMICAL AND STRUCTURAL PROPERTIES OF ULTRAFILTERED MILK

by

SOMJAI SRILAORKUL ©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

IN

DAIRY SCIENCE

DEPARTMENT OF FOOD SCIENCE

EDMONTON, ALBERTA

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ABSTRACT

The effect of ultrafiltration (UF) of skim milk on the physical, chemical, biological and structural properties were studied.

The effect of UF on buffer capacity and composition contributing to the buffer system intensity in skim milk retentate was determined. The concentration of fat, casein and whey proteins in UF retentate increased in direct proportion to the concentration factor (CF) whereas the concentration of Ca, P, Mg, K and Na as well as the maximum buffer capacity increased linearly with CF.

The effect of UF on growth and activity of starter cultures in UF milk retentates was investigated. The use of large inocula in order to overcome the buffer capacity effects resulted in an uncoupling of growth from lactic acid production in all strains studied. The activity and inoculum size required to give the desired pH change in UF milk for cheese quality under simulated cheese manufacturing conditions is presented.

The UF process caused significant changes in casein micelle size distribution. The maximum proportion of casein micelles was changed from 80-100 nm in diameter in skim milk to 60-80 nm in diameter in 5-fold concentrate. As CF was increased the numbers of casein micelles with diameter less than 80 nm increased while those with diameter larger than 100 nm decreased. Consequently, the volume distribution and the average diameter of the casein micelles were altered. There was a highly significant decrease in micelle diameter from 118 nm in skim milk to 92 and 87 nm in 3-fold and 5-fold concentrates, respectively ($P < 0.01$). The change in micelle size distribution, volume distribution

and average diameter of the casein micelles as a result of change in milk compositon from UF process may influence the processing and rheological properties of the UF milk and cheese.

Microstructure of rennet gels derived from the UF milk retentate exhibited slow changes in microstructure as a function of time elapsed after coagulation time (RCT). All acid gels obtained from the same UF retentates reached the end point structure rapidly. Once the end point structure had been reached no further change in gel structure occurred as a function of time. As a result, the network of all acid gels obtained were similar to those of rennet gels at 60 min after RCT. However, the acid gel showed a denser network structure, smaller pores and smaller casein micelle size than rennet gels of the same CF at 60 min after RCT. These differences in microstructure and the way the micelles are aggregated may be responsible for the overall difference in physical properties between acid and rennet gels. As the milk concentration increased, the network structure of both acid and rennet gels became denser with decreased pore and micelle size. Reduction in mean free distance of casein micelles restricts movement and position of the casein micelles with stronger links in the casein network which may influence the final cheese texture. Rennet gels derived from 3-fold and 5-fold UF retentates exhibited curved cracks or narrow void spaces throughout the gel network at RCT which were more pronounced in the 5-fold concentrate. However, 30 min after RCT these curved cracks disappeared. These curved cracks may indicate that the amount of casein micelles incorporated into the curd at RCT decreases with increased casein concentration.

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1. INTRODUCTION

Commercial application of ultrafiltration (UF) in the food industry has increased principally as the result of the following advantages: its high selectivity, low energy requirement compared with the conventional system and little or no detrimental change in nutritional, functional and sensory qualities of food products (Hedrick, 1983-84). In the dairy industry, the use of UF is more predominant than other food industries and the main application of UF technology is in cheese manufacturing (Hollstrom and Tragardb, 1982)

1.1 Advantages of Application of UF for Cheese Manufacturing

The principal application of UF process in cheesemaking is to include whey proteins into the cheese. This results in increases in cheese yield and nutritive value as well as reducing the whey disposal problem and at the same time more efficient utilization of whey proteins than ever before (Mortensen, 1984; Brown and Ernstrom, 1982). Traditionally cheese is manufactured by coagulating the casein to form curd in which fat is entrapped. Then the two components: casein and fat are concentrated by removal of whey which involves many tedious processing steps: cutting, cooking, stirring, whey draining and curd pressing. The whey contains whey proteins, lactose and minerals. If the milk is concentrated first by ultrafiltration to a composition similar to that of the desired cheese, called "precheese", not only casein and fat but also whey proteins (20% of total milk proteins) are included. The liquid precheese is transformed into cheese by adding rennet and starter culture and is then molded, without whey drainage. The most important factor improving cheese yield is the retention of milk solids. This is achieved in a UF process. Therefore only lactose and soluble minerals are removed with the permeate. In addition, the UF process offers a number of technical and economic advantages: the need for whey drainage is eliminated, thus fat and fines loss are minimized, product consistency is improved, the continuous process can be applied, manpower and energy requirement is minimized, processing time and space are reduced, thus greater efficiency is achieved, and saving of rennet up to 85% (Maubois and Mocquot, 1975; Kosikowski, 1986).

1.2 Problems Involved in Application of the UF Process for Manufacturing of Hard Cheese Varieties

UF process has been used with extensive economical success mainly for the manufacture of soft cheese varieties which have low solids content (< 40%) (Scott, 1986; Cheryan, 1986b). However, for the manufacture of hard cheese varieties with higher solids content, the advantages of the UF process are less obvious. Chapman et al. (1974) reported that quality of Cheddar cheese made from UF milk to two-fold concentrate, followed with the traditional cheesemaking procedure was satisfactory but cheese yield was not improved. Furthermore, UF cheesemaking presents many major problems. Attempts to use more highly concentrated UF milk to produce hard cheeses resulted in slight yield improvement but poor quality: atypical texture and flavor (Kosikowski, 1986; Fox, 1984).

The technological problems of UF cheesemaking can be summarized as follows:

1.2.1 Increase in buffer capacity of highly concentrated milk

This makes it very difficult to achieve the optimum pH for good quality cheese. Failure to reduce pH increases the risk of growth of spoilage and pathogenic organisms (Kosikowski, 1986). Much acid is required to reduce the pH of cheese to the optimum region which is very important since it influences (Lawrence et al., 1984; Fox, 1984):

- Retention of coagulant in the curd.
- Activity of the coagulant.
- Solubilization of colloidal calcium phosphate and consequently the concentration of calcium in the curd which in turn influences curd structure and consequently cheese texture.
- Activities of coagulants, plasmin and starter culture enzymes during cheese ripening.

1.2.2 High content of minerals

The content of minerals associated with casein micelles increases as milk concentration is increased. This causes acid/bitter taste and sandy texture in UF cheese (Maubois, 1980; Glover, 1985).

1.2.3. Coagulation of UF Concentrated milk

Gel formation of UF concentrated milk occurs when only a fraction of the micellar k-casein has been hydrolysed as indicated by the proportion of non-protein nitrogen (NPN) released. The proportion of NPN released decreases with increasing milk concentration (Fox, 1984). Thus the proportion of casein incorporated into the curd at coagulation time decreases as milk concentration is increased (Dalgleish, 1981). This results in less developed structure of the UF curd and may be responsible for the unsatisfactory texture in UF hard cheeses (Fox, 1986).

The structure of the UF curd becomes coarser as the milk concentration is increased. If the coarse protein structure is formed during the curd formation, the coarse structure is maintained throughout the chesemaking procedure until the cheese matures. The structural difference may be due to the difference in concentration of milk components. The high casein content may be mainly responsible for the coarse structure of the curd (Green et al., 1981b). In addition, a difference in the mechanism of casein aggregation in higher UF concentrated milk may influence the curd structure as well, consequently affecting the UF cheese structure (Dalgleish, 1981; Green et al., 1981b).

1.2.4 Calcium to casein ratio in UF Cheese

The ratio of calcium to casein is an important parameter to determine the basic structure and therefore the texture of the cheese (Lawrence et al., 1983). In traditional cheese, the calcium to protein ratio is dependent only upon the extent of acid production in the cheese vats. However, it is difficult to control the calcium to protein ratio in UF cheesemaking since it involves 4 distinct stages in the process: preacidification of milk, ultrafiltration, diafiltration and acid production in the coagulum (Jensen et al., 1988).

1.2.5 Influence of whey proteins on properties of UF cheese

The presence of whey proteins in UF cheese may influence the textural properties and ripening of the cheese. This has been a subject of debate. Some reports indicated that if the mineral content of UF cheese is controlled, the organoleptic properties of UF cheese is identical to that made by conventional cheesemaking process (Olsen, 1984) and whey protein incorporation does not affect ripening (Korolczuk et al., 1986). However, others have reported that the presence of whey proteins resulted in softer and smoother texture in UF cheese (De Koning et al., 1981; Van den Berg, 1979). Lawrence (1989) suggested that the effects of whey proteins on UF cheese depends upon the proportion of whey proteins incorporated and the state of undenatured and denatured whey proteins.

Development and introduction of UF membranes, polysulfone and mineral membranes have made it possible to ultrafilter milk at 50°C or higher (Cheryan, 1986a). Denaturation of whey proteins depends upon the processing conditions applied during UF: temperature, the extent to which UF milk is recycled, the residence time and shear denaturation of whey proteins which occurs when the concentrate is pumped through valves during ultrafiltration (Lawrence, 1989). The proportion of air entrapped may cause denaturation of whey proteins, particularly β-lactoglobulin, which is readily denatured by an air-liquid surface effect (Reese and Robbins, 1981).

i. Effect of undenatured whey proteins on UF cheese

Undenatured whey proteins are resistant to the proteolytic activities of rennet, plasmin (indigenous milk alkaline proteinase) and starter cultures (De Koning et al., 1981; Jost et al., 1976). Incorporation of whey proteins results in a cheese with a soft texture. Since the strength and firmness of traditional cheese is due mainly to the casein framework, inclusion of 20% undenatured whey proteins would affect the texture of UF cheese by acting as an inert filler (De Koning et al., 1981).

Undenatured whey proteins may indirectly influence cheese ripening by a dilution effect, thus lowering the proportion of casein in the UF cheese. The whey proteins may physically interfere with the accessibility of the casein to the enzymes which cause ripening. Alternatively these enzymes may be less mobile in cheese containing substantial quantities of whey proteins, which leads to increased viscosity in highly concenatrated UF milk (Lawrence, 1989). Because proteolytic breakdown of casein during cheese ripening is essential for characteristic texture and flavor development, the inclusion of undenatured whey proteins therefore may influence the properties of the UF cheese.

ii. Effect of denatured whey proteins. Denatured whey proteins could influence the UF cheese in several ways:

- Denatured whey proteins are hydrolyzed during cheese ripening which results in the liberation of H_2S and other sulfur compounds from the sulfur rich whey proteins. These flavorful compounds could be responsible for the flavor defect in the UF cheese (Brown and Ernstrom, 1982).
- Inhibition of plasmin by β-lactoglobulin may also occur (Visser, 1981). Plasmin plays a limited but important role in conventional cheese ripening and is mainly responsible for the proteolytic breakdown of α- and β-casein. Thus the presence of denatured whey proteins in UF cheese inhibits β-casein and α_s-casein degradation by plasmin, consequently the UF cheese ripens at a slower rate than in the traditional cheese and results in lack of flavor and atypical texture in UF cheese (Lawrence, 1987).

Denatured whey proteins may also influence UF cheese ripening by the same indirect methods that apply to undernatured whey proteins (Lawrence, 1989).

1.2.6 Lack of stretchability and meltability of UF Mozzarella type cheeses.

The optimum stretchability and meltability of traditional mozzarella is obtained at pH 5.2 (Guinee and Fox, 1987). However, the mechanisms involved in achieving the desired characteristics are not completely understood. It appears that the ratio of initial casein to colloidal calcium and the ratio of casein to moisture determine both stretchability and meltability of the cheese (Lawrence, 1987). Therefore any change in proportions of casein, calcium and moisture will markedly affect these properties. The lack of stretchability and meltability in UF mozzarella may be due to the incorporated whey proteins which affect the casein matrix by binding to para-k-casein and α_{s2}-casein through disulfide interchange to form casein-whey protein complexes. This binding may hinder stretching and melting properties. Another possibility is that the denatured whey proteins compete with casein for binding with calcium ions, thus reducing stretchability and meltability of the UF cheese (Lawrence and Gilles, 1986). Alternately the binding of moisture by denatured whey proteins may decrease meltability of the UF cheese (Sood and Kosikowski, 1979). So, as increasing amounts of the whey proteins are incorporated in the UF cheese, the poorer would be the stretchability and meltability. Since the mechanisms involved are not completely understood at present little can be done to ensure the manufacture of UF Mozzarella type cheese with good stretchability and meltability (Lawrence, 1989).

1.3 Objectives

Although buffer capacity plays a significant role in UF cheese, there is no information available on which component or its proportional contribution to the buffer capacity of UF milk, at high concentration. There is a lack of information on performance of starter culture and inoculum size required to obtain the optimum pH for cheese quality; and the effect of UF process on size distribution of casein micelles. Since acid and rennet coagulation are the two major methods used in traditional cheesemaking, it is necessary to examine the microstructure of gel formation obtained from both methods in relation to their influence on rheological properties of the UF cheese. The objectives of these studies were as follows:

i. to determine the effect of the UF on chemical composition, buffer capacity and buffer system component in skim milk.

ii. to determine the growth and activity of starter cultures in UF skim milk and the size of inoculum required in UF cheesemaking.

iii. to determine the effect of UF on casein micelle size distribution, average diameter and volume distribution of the casein micelles in skim milk.

iv. to investigate the effect of UF on microstructure of gels obtained from acid and rennet coagulation.

This thesis is presented in the paper format following guidelines of the Faculty of Graduate Studies and Research, University of Alberta. The papers presented are condensed forms, therefore this chapter provides detailed background information for the subjects involved.

1.4 Ultrafiltration

Ultrafiltration (UF) is a pressure driven membrane process for fractionation, clarification and selective concentration of components in an aqueous system.

The principle of UF is described as a pure sieving process through a membrane which has definite pore size. The feed flows parallel to the surface of the membrane, i.e. crossflow filtration (Figure 1.1) which results in higher efficiency of permeation flow than the dead end filtration where the direction of the flow is perpendicular to the filter membrane (Shannon, 1987). The smaller molecules which are responsible for the osmotic pressure pass through the membrane as permeate while larger molecules, suspension solids and emulsion droplets are retained as retentate (Jameson, 1984). Therefore UF is not concerned with osmotic pressure and as a result the working pressures of UF are very low, in the range of 500 kPa (5 bar) (Glover, 1985). The ability of membrane to retain the smallest defined molecule of known molecular weight is generally used to specify the porosity of the membrane in term of "molecular weight cut-off".

1.4.1 Performance of the membrane

Important considerations for determining the technical feasibility of UF are membrane performance, the flux rate and retention factor (Glover, 1985).

i. Flux rate is the rate of solution or permeate transport through the membrane, it is defined as:

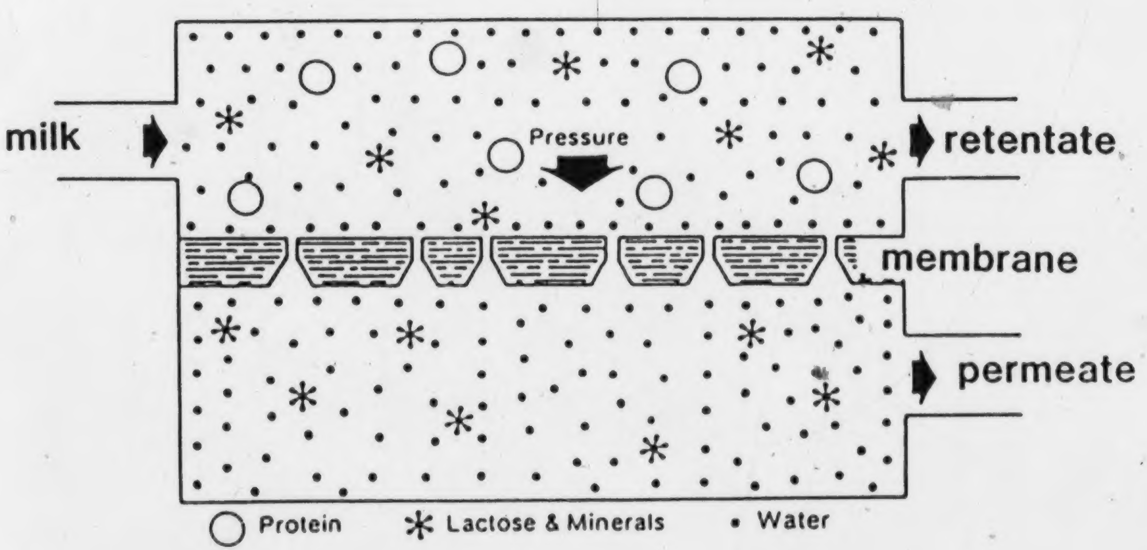
milk
Pressure
retentate
membrane
permeate
Protein
Lactose & Minerals
Water

Figure 1.1 Diagram of the ultrafiltration membrane process.

$$\text{Flux rate} = \frac{\text{Liter of permeate produced}}{\text{m}^2 \text{ of membrane surface - h}}$$

ii. Retention factor. Because UF is used to concentrate and to retain the component of value, membrane retention factor specifies the separation efficiency or ability of the membrane to retain the desired component and is expressed as:

$$RF = \frac{Cf - Cp}{Cf}$$

Where RF is retention factor, Cf is concentration of the molecules in the feed, and Cp is concentration of the molecules in the permeate.

1.4.2 Types of membranes for ultrafiltration

The success of milk UF principally depends upon the performance and the reliability of the UF membrane (Mocquot, 1979). Various types of membranes have been developed rapidly with improvement of mechanical, thermal and chemical stabilities to meet the requirement of food industrial use and to comply with strict regulations that control process equipment in contact with food material. Further, the systems have to be perfectly cleaned and sanitized to avoid any possible contamination in the food process (Paulson et al., 1984). Types of membrane available commercially are:

i. Cellulose acetate membranes

The first commercially developed UF membrane was the cellulose acetate membrane. These membranes are prepared from cellulose by acetylation with acetate, the structure of cellulose acetate is given in Figure 1.2.a.

There are several advantages of cellulose acetate membranes such as high flux and high selectivity. The membranes are easy to manufacture and the material is available at low cost. However, the membranes are easily damaged by changes in temperature and pH. Since they are esters of polysaccharide they hydrolyze under acid conditions, on the other hand, highly alkaline conditions cause deacelytation. Thus, the membranes have poor thermal and chemical stabilities. They can only operate at maximum temperatures of 30°C and at pH range of 3-7. Further, cellulose acetate membranes are poor in mechanical strength, they undergo creep on compaction, thus losing membrane performance under pressure (Cheryan, 1986a).

ii. Polysulfone membranes

Due to the severe limitation of cellulose acetate membranes, polysulfone membranes have been developed and they are replacing cellulose acetate membranes. Polysulfone contains repeating units of diphenyl sulfone, $[(C_6H_5)_2SO_2]$ in its structure (Figure 1.2b). Repeating phenyl units provide steric hindrance to rotation within the molecules thus contributing to a high degree of molecule immobility and consequently a membrane of high mechanical strength and resistance to creep. Polysulfone membranes are capable of withstanding temperatures up to 80°C and a pH range of 2-12 (Coton, 1986). The membranes also have better resistance to chlorine and to compaction than cellulose acetate membranes. Further, the membranes are easy to fabricate in a variety of configurations and in a wide range of UF applications ranging from molecular weight cut-off from 1,000 to 500,000 daltons.

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Figure 1.2 Structure of cellulose acetate (A) and polysulfone (B) (Cheryan, 1986; Glover, 1985).

Therefore polysufone membranes are presently used in most UF plants (Cheryan, 1986a).

iii. Mineral or ceramic membranes

The most recent membranes commercially available are mineral or ceramic membranes. Zirconium or aluminum oxide is the active part of the membrane and porous carbon or porous ceramic is the membrane support in form of tubes. These membranes offer advantages over polysulfone because of their great mechanical strength. They are able to withstand pressures up to 4000 kPa (40 bar) and they have the ability to tolerate the entire pH range and temperatures of up to 400°C. The only drawback of these membranes is their cost (Banks and Muir, 1985; Kosikowski, 1986).

1.5 Chemical Composition of Milk, Physical States of Milk Components and Technical Importance of Milk Proteins.

1.5.1 The chemical composition and physical states of milk

The compositon and physical states of milk make it very suitable for membrane fractionation and concentration. Milk is a complex fluid containing many compounds in various sizes and states of dispersion (Jenness and Patton, 1959). The composition of milk is illustrated in Table 1.1. The major constituents of milk are: water present as continuous phase, fat in emulsion droplets, casein in colloidal dispersion while whey proteins, lactose and part of minerals are in true solution. Part of the minerals is associated with casein in colloidal form. These components vary in size from molecular weight of 18 for water, 60 up to 300 for minerals, 342 for lactose, 15,000 upward

Table 1.1 Chemical composition and physical states of milk:

	Constituent	Content
1.	Water in liquid continuous phase, %	87.00
2.	Lipids in emulsion phase	
	a. Milk fat (triglyceride), %	3.80
	b. Phospholipid, %	0.02
	c. Carotenoids, mg/100g	0.04
	d. Vitamins A, D, E and K, mg/100 g	0.02
3.	Proteins	
	a. Casein colloidal dispersion, %	
	αs_1-casein	1.00
	αs_2-casein	0.26
	β-casein	0.93
	κ-casein	0.33
	γ-casein	0.08
	b. Whey proteins in solution, %	
	β - lactoglobulin	0.32
	α - lactalbumin	0.12
	Blood serum albumin	0.04
	Immunoglobulin	0.08
	Proteose - peptone & others	0.06
	c. Enzymes	
	Lactoperoxidase and triacylglycerol lipase in milk serum	
	Acid phosphatase, alkaline phosphatase and xanthine oxidase associated with milk fat globule membrane	
	Plasmin associated with casein micelles	

4.	Lactose in solution, %	4.60
5.	Minerals and organic salts in solution, mg/100g	
	Ca	37.00
	Mg	7.50
	P	134.00
	K	134.00
	Na	46.00
	Cl	106.00
	Citrate	160.00
	Bicarbonate	10.00
6.	Minerals and organic salt in colloidal form, mg/100g	
	Ca	80.00
	P	95.00
	Citrate	14.00
	Mg, K, Na, etc.	15.00
7.	Non-protein nitrogen in solution, mg/100g	
	Peptide	20.00
	Amino acids	30.00
	Urea	30.00
8.	Water soluble vitamins, mg/100 g	
	B vitamins	20.00
	Ascorbic Acid	2.00

Adapted from Walstra and Jenness (1984); Jenness & Patton (1959) for proteins and fat present in large globules with diameters of 10^2-10^4 nm (0.1-10 μm) (Table 1.2). These properties make milk very suitable for UF processing, for fractionation and concentration of the desired component. In whole milk up to 99% protein and 100% of fat retention can be achieved by UF process (Cheryan, 1986a).

1.5.2 Milk proteins

Milk contains approximately 3.3% protein. About 80% of the protein consists of casein, a group of phosphoproteins which precipitate upon acidification of milk to pH 4.6 at 20°C. The proteins that remain soluble are whey proteins (De Wit, 1981).

i. Caseins

Caseins consist of 4 principal proteins: α_{S1}-, α_{S2}-, β- and k-casein in the approximate average weight ratio of 3:0.8:3:1 (Schmidt, 1982). In addition there are several proteins which originate from post secretion proteolysis of primary casein by plasmin, these include: γ-caseins and proteose-peptone components 5, 8 fast and 8 slow all derived from β-casein; and λ-casein derived from α_{S1}-casein (Fox and Mulvihill, 1982). The primary structure of the 4 major caseins α_{s1}-, α_{s2}-, β- and k-caseins are presented in Figures 1.3, 1.4, 1.5 and 1.6, respectively.

The most important characteristics of technical importance in these primary structures are:

a. The uneven distribution of acidic amino acids, serine phosphate groups and hydrophobic amino acids along their molecules. This leads to an amphiphilic conformation with strongly hydrophilic and hydrophobic regions which render caseins highly susceptible to intermolecular interaction through the formation of hydrophobic

Table 1.2 Molecular size of milk components

Constituent	Molecular weight	Diameter, nm
Water	18	0.3
Chloride ion	35	0.4
Calcium ion	40	0.4
Lactose	342	0.8
α-Lactalbumin	14,500	3
β-Lactoglobulin	19,000	4
Blood serum albumin	69,000	5
Casein micelles	10^7 - 10^9	20 - 600
Fat globules	-	10^2 - 10^4

Adapted from Glover (1985)

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Figure 1.3 Primary structure of α_{s1}- casein B-8P (Whitney, 1988).

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Figure 1.4 Primary structure of α_{s2}-casein A-11P (Swaisgood, 1982).

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Figure 1.5 Primary structure of β-casein A^2-5P (Whitney, 1988).

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Figure 1.6 Primary structure of k-casein B-1P, the A variant has a threonine at position 136 and an aspartic acid residue at position 148.
The arrow indicates the point of attack of rennin (Whitney, 1988).

interactions and ionic bonds, and to function as emulsifiers at lipid/water or air/water interfaces (Kinsella and Whitehead, 1986).

b. The high content and uniform distribution of prolyl residues along their molecules. Random coils or open structures with little secondary structure occur because prolyl residues do not participate in the formation of α-helix and β-sheet structures in proteins (Swaisgood, 1982). Therefore the caseins are not subject to heat denaturation during the heat processing of milk. However, the open structure renders them more susceptible to hydrolysis by the proteolytic enzymes, rennet, plasmin and starter culture which are important in cheesemaking (Morr, 1975).

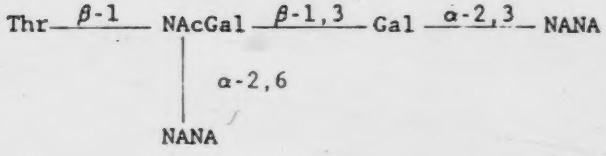
c. Only k-casein and α_{s2}-casein contain cysteine groups, each has 2 cysteine residues. The sulfhydryl cysteine residues are responsible for self association of k-casein and α_{s2}-casein. They are also capable of interacting with β-lactoglobulin during heat processing by disulfide interchange mechanisms at or above 65°C. This has a detrimental effect upon the cheesemaking properties of milk (Morr, 1979).

d. Different levels of phosphorylation. All caseins are phosphorylated in different levels : α_{s1}-, α_{s2}-, β- and k-casein contain 8-9, 10-13, 5 and 1 phosphate residues per molecule, respectively. All phosphorylated residues in the casein are serines with the exception of α_{s1}-D casein. In α_{s1}-D casein, one additional threonine is also phosphorylated (Swaisgood, 1982). Because of their high phosphate content, α_{s1}-, α_{s2}- and β-caseins bind Ca^{2+} strongly and precipitate. The degree of calcium-sensitivity is directly proportional to the phosphate content of the casein. Thus k-casein which has only one phosphate residue in the polar domain C-terminal portion of the molecule is not sensitive to Ca^{2+}. Furthermore, k-casein can associate with α_{s1}-, α_{s2}- and β-casein, which are calcium sensitive caseins, and it can stabilize them from calcium ion-induced precipitation (Swaisgood, 1973).

e. Glycosylation of k-casein. The only glycoprotein in the milk caseins is k-casein. Its carbohydrate moiety consists of N-acetylneuraminic acid (NANA), galactose and N-acetyl-galactosamine (NAc Gal). These residues usually exist as a trisaccharide as:

$$\mathrm{Thr}\overset{\beta\text{-}1}{\rule{3em}{0.4pt}}\mathrm{NAcGal}\overset{\beta\text{-}1,3}{\rule{3em}{0.4pt}}\mathrm{Gal}\overset{\alpha\text{-}2,3}{\rule{3em}{0.4pt}}\mathrm{NANA},$$

or as tetrasaccharide:



$$\mathrm{Thr}\overset{\beta\text{-}1}{\rule{3em}{0.4pt}}\underset{\substack{\Big|\ \alpha\text{-}2,6\\ \mathrm{NANA}}}{\mathrm{NAcGal}}\overset{\beta\text{-}1,3}{\rule{3em}{0.4pt}}\mathrm{Gal}\overset{\alpha\text{-}2,3}{\rule{3em}{0.4pt}}\mathrm{NANA}$$

(Fox and Mulvihill, 1982).

These oligosaccharides are attached to the molecules mainly at threonine 133, but also at threonine 131 and threonine 135. Therefore the number of oligosachharide chains can vary from 0 to 3. Glycosylation of k-casein increases the negative charge of the hydrophilic C-terminal portion by virtue of the negatively charged oligosacharide residues (Swaisgood, 1973). Consequently, the stabilizing properties of k-casein are increased (Walstra and Jenness,1984).

ii. Casein micelles

The casein in milk occurs in the form of a colloidal dispersion, called casein micelles which is responsible for the turbidity of milk. Processing properties of milk (pasteurization, sterilization, concentration and enzymatic coagulation in cheesemaking) are strongly influenced by the stability of casein micelles (Schmidt, 1982). The micelles are roughly spherical, ranging in size from 20-600 nm (Schmidt, 1982). On a dry weight basis the micelles consist of approximately 94% protein and 6% colloidal calcium phosphate (CCP) containing principally calcium, phosphate, magnesium and citrate. Although CCP is present in low concentration, it plays a major role in maintaining the integrity of casein micelles (Fox, 1986). The amount of casein varies with size of the micelle. Smaller micelles contain a larger proportion of k-casein (Swaisgood, 1973).

The precise structure of the casein micelle is unknown. Many models of casein micelles have been proposed. The latest model (Schmidt, 1982) proposed submicelles linked together by CCP in the form of $Ca_9(PO_4)_6$ clusters (Figure 1.7). The micelles are assembled with k-casein deficient submicelles as a core surrounded by k-casein rich submicelles to serve to terminate the process. Thus the intact micelles contain a surface rich in k-casein so that k-casein can give a stabilizing effect upon the casein micelles.

Although the structure of the casein micelle is not known with certainty, it is generally accepted that the micelles are composed of submicelles held together by CCP, hydrophobic interactions, hydrogen bonds and electrostatic bonds (Farrell, 1988; Fox, 1986). Evidence from

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Figure 1.7 Casein micelle model proposed by Schmidt (1982). Schematic representation of a submicelle, (A) and a casein micelle composed of submicelles, (B).

labelled k-casein in electron micrographs (Schmidt and Both, 1982) indicated that almost all k-caseins occur at the surface of casein micelles. Labelled α_{s1}- and β-caseins are scattered throughout the whole micelle, including the micelle surface. The hydrophilic C-terminal portions of k-casein protrude into the surrounding environment as "a hairy layer" (Bringe and Kinsella, 1987; Fox, 1986). Therefore the casein micelles are stabilized by steric repulsion caused by protruding C-terminal regions of k-casein and by a zeta potential of about -20 mV of the net negative charge of the micelles, thereby preventing the close approach and aggregation of casein micelles (Dalgleish, 1987; Fox, 1986).

iii. Whey proteins

Whey proteins contain mainly β-lactoglobulin (β-lg) (50%) and α-lactalbumin (α-la)(20%). The remainder is composed of serum albumin, immunoglobulin and proteose-peptones. The primary structures of the 2 major whey proteins: α-la and β-lg are given in Figures 1.8 and 1.9, respectively.

Comparison of the primary structure of these whey proteins with those of caseins indicates some major differences which can be summarized as follows:

a. Whey proteins are typical compact globular proteins with a uniform distribution of acidic/basic and hydrophobic/hydrophilic amino acids. Therefore they lack the amphiphilic nature of the casein molecule (Morr, 1979).

b. Considerably lower proline content in the whey protein molecules permits a substantial amount of helical structure and therefore results in their higher susceptibity to denaturation by heat

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Figure 1.8 Primary structure of α-lactalbumin B (Swaisgood, 1982).

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Figure 1.9 Primary structure of β - lactoglobulin B and other genetic variants. The SH group is postulated to be equally distributed between position 119 and 121, with the -S-S- bridge location depending upon the position of the SH-group (Whitney, 1988).

treatment (Morr, 1979).

c. Whey proteins contain no esterified phosphate residues in their molecules (Swaisgood, 1982).

d. The high content of sulfhydryl and disulfide groups in whey protein molecules plays an important role in the interaction with k-casein via intermolecular disulfide bond formation under the influence of heat (Morr, 1975).

1.5.3 Technical Importance of milk proteins

Due to the high content of casein in milk (80% of total milk proteins), casein dominates in determining the characteristics of milk during processing (Brown, 1988). Much dairy technology is concerned with processes that destabilize the casein micelles which is exploited in the production of dairy products (Jenness and Patton, 1959). Some of the processing treatments which cause destabilization of casein micelles and have important application in dairy technology are:

i. Acidification

The casein micelles are very sensitive to change in pH since casein micelles are stabilized and dispersed in the solution by the overall negative charge carried by the micelles on their surface. This is referred to as electrostatic and steric repulsion from the hairy layer at the micellar surface (Fox, 1986; Walstra and Van Vliet, 1986).

The surface charge of casein micelles is measured by the zeta potential which is proportional to the mobility of the charged particles in an electrical field. The zeta potential of casein micelles is about -20 mV (Fox, 1986). As the pH is lowered carboxyl groups of the protein are protonated to their non-ionic form, amino groups are protonated to their cationic form and the electrostatic repulsion is reduced. At pH 5.2 the zeta potential of casein micelles becomes close to zero and the casein micelles start to aggregate (Heertje et al., 1985). The maximum aggregation of casein is at its isoelectric point where the micelles have no net charge and the zeta potential of the casein micelles is zero (Hayakama and Nakai, 1985). The isoelectric point of casein micelles is at about pH 4.6 (Bringe and Kinsella, 1987).

However, the electrostatic repulsion of casein micelles is not enough to stabilize the casein micelles (Dalgleish,1987; Fox, 1986) because hydrophobic colloids with zeta potentials of about -20 to -30 mV are generally unstable. Therefore, the hairy layer (C-terminal end of k-casein containing strongly hydrophilic chains protruding into the surrounding liquid) at the micelle surface may play an important role in stabilizing the micelles through steric repulsion (Walstra and Van Vliet, 1986). The hairy layer of k-casein causes the casein micelles to have high voluminosity, therefore measurements of voluminosity are directly related to steric repulsive forces between casein micelles. As the pH is lowered from 6.5 to 4.5 the voluminosity of the casein decreases (Darling, 1982). This indicates that the coagulation of casein micelles at low pH could possibly be a result of decreased steric repulsion by hydrogen ions. Acid precipitation of casein micelles is exploited in manufacturing of dairy prodcts including acid casein and fermented dairy products such as yoghurt and some varieties of cheeses, e.g. Cottage cheese.

ii. Rennet coagulation of milk

The specific cleavage of the sensitive Phe_{105}-Met_{106} peptide bond of k-casein by rennet and the subsequent coagulation of rennet-altered micelles by Ca^{2+} at temperatures greater than 18°C is the most important process in the initial step of manufacture of most cheese varieties and rennet casein (Walstra and Jenness, 1984).

It is well established that coagulation of milk by rennet is a result of 2 reactions: primary (enzymic) and secondary (nonenzymic) phase.

Primary (enzymic) phase

If milk is clotted effectively, the rennet must attack the k-casein at the Phe_{105}-Met_{106} bond. This cleavage yields para-k-casein which is strongly hydrophobic and associated with the rennet-altered micelles. The cleavage also releases the highly charged hydrophilic macropeptide which contains variable amounts of carbohydrates from the C-terminal end of k-casein (Fox, 1986). The loss of macropeptide results in reduction of the surface potential from -20 mV to about half and loss of steric stability of the micelles, therefore closer approach of the micelles is facilitated (Dalgleish, 1987; Green, 1982). The specific attack of rennet on k-casein is of great importance for milk coagulation because other general proteolysis causes loss of cheese yield by the formation of soluble peptides and bitterness from the hydrophobic peptides (Visser et al., 1975).

All commercial rennets which are acid proteinases are remarkably specific for the Phe-Met bond of k-casein (Dalgliesh, 1987). This specificity may be affected by 2 factors:

a. The sequence or primary structure of the substrate around the sensitive bond is an important determinant of enzyme-substrate interaction (Visser, 1981). The Phe-Met bond by itself is not susceptible to attack by rennet because simple di-, tri- and tetra-peptide methyl esters, even if they contain the Phe-Met bond, are not hydrolyzed at all. It appears that the minimum requirement for the hydrolysis is a chain length of 5 amino acid residues containing Ser-Leu-Phe-Met-Ala-OMe. When the serine and leucine are interchanged to give the same sequence as in k-casein, the hydrolytic efficiency is increased. Extension of this pentapeptide in either direction of the sensitive bond towards the C- or N-terminal end of the protein to reproduce the sequence of k-casein increases the efficiency of hydrolysis of the Phe-Met bond. Taking the pentapeptide Ser_{104}-Ile_{108} of k-casein as a standard (Figure 1.6), extending the chain towards C-terminal by 3 amino acids to give the peptide Ser_{104}-Lys_{111} gives a 6-fold increase in catalytic ratio, k_{cat}/K_m (k_{cat} is catalytic constant and K_m is the affinity of enzyme for the substrate) whereas addition of Leu_{103} to the pentapeptide increases the catalytic ratio about 600-fold. The hydrophobic amino acids (Leu_{103}, Phe_{105}, Met_{106}, Ala_{107}, and Ile_{108}) may contribute to the enzyme-substrate interaction through hydrophobic interactions (Visser et al., 1977). Incorporation of His_{102} and Pro_{101} increases k_{cat}/K_m a further 5-fold. The three histidines (residues 98, 100 and 102) are also important in binding of substrate to the enzyme through ionic bonding because k_{cat}/K_m is about 30 times greater for segment 98-111 than the segment 103-111 which does not contain these histidines. Leu_{103} is also strongly important in binding because k_{cat}/K_m is about 300 times greater for segment 103-111 than for segment 104-111 and the two proline residues at positions 109 and 111 are also strongly involved in the binding (Visser, 1981; Visser et al., 1977). Ser_{104} is essential for the hydrolysis since substitution of Thr for Ser_{104} reduces k_{cat}/K_m about 5-fold while substitution of Ala reduces this ratio approximately 30- to 40-fold. Furthermore a peptide containing D-Ser instead of L-Ser is not hydrolyzed at all by rennet, therefore the β-OH group of Ser is important for enzyme-substrate binding, possibly via a H bond formation (Raymond and Bricas, 1979; Visser et al., 1977). Therefore the sequence around the Phe-Met bond rather than the bond itself determines the specific hydrolysis of the Phe-Met bond.

b. The overall conformation or secondary and tertiary structure of the substrate must be such that the sensitive bond is in the position readily accessible to the enzyme (Dalgleish, 1987).

Many small peptide substrates are structureless since they are too small to fold to give the required conformation. This is not the case in the intact protein like k-casein. Although there is no definite structure available for k-casein, Loucheux-Lefebvre et al. (1978) have proposed that the sequence around the rennet sensitive bond may be capable of forming a β-sheet conformation and is situated between two stable β-turns. This may cause the sequence to project from the k-casein molecule and enables it to fit into the active cleft of the enzyme.

Both natural variations in genetic variants A and B , and the different levels of glycosylation of k-casein have little or no effect on the hydrolytic rate of the Phe-Met bond because the sites of their modification are far from the Phe-Met bond (Dalgleish, 1986).

The primary phase of rennet action is dependent upon:

- Enzyme concentration. The rate of hydrolysis is strongly influenced by enzyme concentration. Castle and Wheelock (1972) reported that the rate of specific hydrolysis of k-casein is directly proportional to the enzyme concentration within a certain limit.

- Temperature. Temperature also influences the rate of hydrolysis. The temperature coefficient (Q_{10}) is about 2 in the temperature range 1 to 30°C (McMahon and Brown, 1984).

- pH. The pH optimum of calf rennet for the specific hydrolysis is in the range 5.0-5.5 and the activity decreases with increasing pH. Extremes of acid or base cause denaturation of the enzyme. Futhermore, pH may affect the affinity of enzyme for the substrate. The fall in activity on either side of the pH optimum may be due to a decrease in affinity (Brown, 1988). However, sufficient hydrolysis by rennet will occur at the natural pH of milk (pH 6.7) to allow clotting of milk (Fox, 1986).

- Ionic strength. The ionic strength of the renneting medium is also important since the enzyme and k-casein are both negatively charged and they repel each other. This electrostatic repulsion is a repulsion between the electrical double layer which surrounds the charged particle. Increasing ionic strength by addition of NaCl or $CaCl_2$, < 10 mM can reduce the repulsion between the enzyme and substrate by direct addition to the electrical double layer and indirectly by binding the negatively charged carboxyl or phosphate groups. This allows close approach and potential formation of an enzyme-substrate complex, thus the rate of hydrolysis increases

(Dalgleish, 1982).

- Pretreatment of milk. Cooling of milk before renneting causes a decrease in rate of hydrolysis as a result of the effect of lower temperature from the optimum temperature of the enzyme (McMahon and Brown, 1984).

- Heating that causes denaturation of whey proteins. Denatured whey proteins, particularly β-lactoglobulin, may reduce the susceptibility of k-casein to attack by rennet as a result of disulfide complex formation between k-casein and β-lactoglobulin. Alternatively, the enzyme may cleave the k-casein but the rennet-altered micelles may not be able to clot because the denatured β-lactoglobulin binds to their surfaces (Hooy Donk et al., 1987; Wheelock and Kirk, 1974).

- Homogenization of milk. Homogenization of milk causes disruption of fat globules into smaller units and adsorption of casein micelles at the newly created globule surfaces (Henstra and Schmidt, 1970). Electron micrographs of homogenized milk show a size distribution of casein micelles similar to that of unhomogenized controlled milk, indicating that homogenization causes no appreciable disruption of the casein micelles (Green et al., 1983). Therefore the micelles are relatively intact when bound to the fat globule surface and there is no significant effect of homogenization of milk on the hydrolytic rate of k-casein (Dalgleish, 1987).

Secondary (nonenzymic) phase

Hydrolysis of k-casein during the primary phase of rennet action with release of the highly charged, hydrophilic macropeptide (residues

106-169) not only removes the electrostatic and steric repulsive forces but also leaves a strongly hydrophobic para-k-casein on the micelle surface. This enhances the hydrophobic interactions leading to the aggregation of rennet-altered micelles (Fox, 1986; Slattery, 1979). However, the proteolysis of a small number of k-casein molecules cannot affect the aggregation and coagulation properties of the micelles because the micelles contain a large number of k-casein molecules. Coagulation of the casein micelles can only occur when about 85% of the total k-casein in milk has been hydrolyzed (Dalgleish, 1979).

There are many factors influencing the rate of aggregation of rennet-altered casein micelles. These factors include:

- Concentration of Ca^{2+}. Ca^{2+} is absolutely necessary for gel formation. Ca^{2+} may contribute to charge neutralization or possibly participate to form calcium bridges, i.e. cross-linking of casein micelles via serine phosphate or carboxyl groups and thus enhance the effective hydrophobic interactions for coagulation (Mehaia and Cheryan, 1983). The rate of coagulation of rennet-altered micelles increases with increased Ca^{2+} concentration from 2.5 to 5 mM except at high temperature (40°C) where the coagulation rates become independent of Ca^{2+} concentration. The lack of an effect of Ca^{2+} at 40°C on the rate of rennet-altered casein micelle aggregation may be due to increased hydrophobic interactions of the micelles at higher temperture (Dalgleish, 1983).

Colloidal calcium phosphate (CCP) is also important in coagulation. When 20% of CCP is removed, rennet-altered micelles cannot coagulate unless Ca^{2+} levels are increased. This may be due to an increase in micellar negative charge when CCP is removed from the micelles (Fox, 1986).

- Temperature. The coagulation of rennet-altered micelles is very temperature dependent. The Q_{10} value of the coagulation for the temperature range 20 to 50°C is about 12 (McMahon and Brown, 1984). Low temperatures (< 18°C) allow the specific hydrolysis of k-casein to be complete in the absence of coagulation (Fox, 1982). Coagulation can occur by subsequent warming. The high temperature dependence of the secondary phase permits continuous curd preparation. The strong effect of temperture on coagulation of the rennet-altered micelles indicates that hydrophobic interactions play an important role in micelle aggregation and gel formation (Kowalchyk and Olsen, 1977).

Temperature not only affects the coagulation of rennet-altered micelles by influencing the driving force for hydrophobic interactions, but also influences the association of β-casein, Ca^{2+} and CCP with the micelles. The inhibition of clotting at low temperature may be partly due to steric repulsion at the surface of the micelles resulting from the incomplete loss of β-casein. At lower temperatures β-casein may dissociate incompletely and possibly stay attached by the C-terminal to the micelle surface and cause steric repulsion between the micelles (Walstra, 1979; Pierre and Brule, 1981).

The dependence of rennet-altered micelle coagulation on temperature is also caused by changes in Ca^{2+} binding to casein. As temperature is lowered the binding of the Ca^{2+} to casein decreases (Dalgleish, 1983).

CCP is solubilized from the micelles at lower temperatures therefore the amount of CCP may not be sufficient for aggregation at low temperatures. However, addition of Ca^{2+} will increase CCP and may cause the cold rennet-altered micelles to coagulate (Pierre and Brule, 1981).

- pH. pH also influences coagulation of rennet-altered micelles which involves micellar charge. When pH is decreased from 6.8 to 6.3 the coagulation rate increases by a factor of about 2 (Kowalchyk and Olsen, 1977). However, the effect of pH on coagulation rate of rennet-treated micelles is rather small as a result of increased concentration of Ca^{2+} when the pH is lowered but, at the same time more CCP is dissolved. Therefore, these effects tend to cancel each other out to give only a small pH influene on the coagulation (Snalabi and Fox, 1982).

Overall kinetics of the enzymatic coagulation of milk

Since the hydrolysis of k-casein by rennet is a single step reaction, it follows Michaelis-Menten kinetics (Dalgleish, 1982, 1987):

$$v = \frac{dP}{dt} = \frac{V_{max}(S_0 - P)}{K_m + S_0 - P} \qquad (1.1)$$

Where v is the initial rate of conversion of substrate into product, V_{max} is the maximum rate at infinite concentration of substrate, K_m is Michelis Menten constant and S_0 and P are concentrations of substrate at time = 0 and product at time = t, respectively. Integration of equation 1.1 will give:

$$\int_0^t V_{max}\, dt = \int_0^P \frac{S_0 - P + K_m}{S_0 - P} .dP \qquad (1.2)$$

$$V_{max}\, t_{pro} = K_m \ln \frac{S_0}{S_0 - P} + P \qquad (1.3)$$

$$t_{pro} = \frac{K_m}{V_{max}} \ln \frac{S_0}{S_0 - P} + \frac{P}{V_{max}} \qquad (1.4)$$

or $$t_{pro} = \frac{K_m}{V_{max}} \ln \frac{1}{1 - \alpha_s} + \frac{\alpha_s S_0}{V_{max}} \qquad (1.5)$$

Where α_s is the critical degree of conversion and equal to P/S_0.

It is well known that during the specific proteolysis of k-casein, the rennet-altered micelles do not aggregate appreciably until approximately 85% of the total k-casein has been hydrolyzed. Above this critical point, the aggregation of the rennet-altered micelles increases linearly with time (Dalgleish, 1979, 1982).

The time required for aggregation of renneted-altered micelles occurs via the von Smoluchowski mechanism, i.e. the growth of weight-average molecular weight is linear with time:

$$M_c = M_0(1 + K_s C_0 t) \qquad (1.6)$$

Where M_0 and M_c are the molecular weights of the aggregating units at time 0 and at clotting time, respectively, C_0 is the concentration of micelles, k_s is rate constant for aggregation. Thus the time for aggregation (t_{agg}) to the average molecular weight at which clotting is first observed will be:

$$t_{agg} = \frac{1}{K_s C_0} \left(\frac{M_c}{M_0} - 1 \right) \qquad (1.7)$$

Rennet clotting time (t_c) is the sum of the time required to give critical proteolysis (t_{pro}) and the time (t_{agg})for the amount of rennet-altered micelles to aggregate, therefore:

$$t_c = t_{pro} + t_{agg} \qquad (1.8)$$

$$= \frac{K_m}{V_{max}} \ln \frac{1}{1 - \alpha_s} + \frac{\alpha_s S_0}{V_{max}}$$

$$+ \frac{1}{k_s C_0} \left(\frac{M_c}{M_0} - 1 \right) \qquad (1.9)$$

Equation 1.9 is similar to the empirical formula:

$$t_c = \frac{A}{V_{max}} + B \qquad (1.10)$$

Where A is constant and B is the time required for the coagulation of the rennet-altered micelles. Since V_{max} is a function of enzyme concentration [E], thus:

$$t_c = \frac{A}{[E]} + B \qquad (1.11)$$

1.6 Cheese Texture and Flavor

6.1 Classification of cheese varieties

Cheeses can be differentiated by the calcium content of their nonfat salt-free solids and pH. Therefore it is best to classify cheese varieties by their calcium content and pH (Lawrence and Gilles, 1987). The four basic types of cheese can be used to represent most of the common cheese varieties, from the very acid Cheshire to low acid Swiss cheese (Figure 1.10).

Swiss, Gouda and Cheshire cheeses have narrow ranges of pH and calcium whereas Cheddar cheese has a relatively wider range because the calcium content and pH levels of Cheddar cheese could vary considerably and still could be within an acceptable range of Cheddar characteristics. Characteristics of Gouda cheese fall between Swiss and Cheddar cheeses in texture characteristics therefore small eyes (holes) in Gouda is expected, whereas no eyes are found in Cheddar. Scanning electron micrographs of these cheese types (Hall and Creamer, 1972) illustrated the distinct change of the protein matrix. The structural units in the protein matrix of Swiss or Gouda cheeses which are high in calcium content and high pH have the same spherical forms as in the

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Figure 1.10 The classification of traditionally manufactured cheese varieties by means of their characteristic ranges of calcium/solids-not-fat ratio and pH (Lawrence and Gilles, 1987).

original cheese milk. On the other hand, in Cheshire cheese with low calcium content and pH the sperical form in the protein matrix is lost. Cheddar cheese is intermediate between Gouda and Cheshire, and the protein matrix in Cheddar is in small spherical forms.

1.6.2 Texture and flavor characteristic of cheese

The differences in characteristic texture and flavor between various types of traditional cheese are fundamentally related to differences in the following:

- Basic structure: Differences in the texture of the various cheese varieties are essentially related to differences in basic structure. The basic structure of cheese is influenced by the point at which curd and whey are separated. This largely determines the mineral content and residual lactose content of the curd, which in turn determines the lowest pH that a cheese can attain. The mineral content of a cheese is determined by the amount of colloidal calcium phosphate (CCP) lost from the curd at draining which is mainly dependent upon the acidity developed before the whey is drained off. The level of loss of CCP, in turn, determines the extent of disruption of the casein micelles and therefore the basic structure of the cheese. Swiss cheese which has low acidity at whey draining will retain higher CCP in the curd resulting in micelle structure similar to that of the cheese milk. Cheshire cheese with low CCP content due to high acidity at draining, results in disruption of casein micelles (Lawrence et al., 1983; Hall and Creamer, 1972).

- Development of chemical composition in the cheese curd: salt to moisture ratio, moisture to casein ratio and pH after salting are important to encourage the microbial and enzymic activities necessary to produce the desired characteristic texture and flavor of the cheese during ripening (Figure 1.11) (Cooper, 1987; Green et al., 1981a; Lawrence et al., 1983).

Microstructure of cheese shows that all cheese varieties contain three major constituents: casein, fat and water. Casein is present as a continuous matrix, fat globules are in dispersed phase while water is partly bound to the protein and the remainder fills the interstices between the casein matrix and the fat (Prentice, 1987). The casein matrix consists of an extensive network of α_{s1}-casein micelles which can form strong interactions with more casein molecules either α_{s1}- or β-casein. Therefore, α_{s1}-casein acts as a link in the casein network (Creamer and Olsen, 1982).

1.6.3 Important reactions in cheese ripening

The characteristic texture and flavor of each cheese variety developed during ripening is due to a complex series of proteolytic, glycolytic and lipolytic reactions catalyzed by rennet, milk enzymes (plasmin and lipase) and enzymes from the primary and secondary starter cultures (Green and Manning, 1982; Wong, 1974).

i. Proteolytic degradation of casein

Proteolytic degradation of casein during cheese ripening is almost entirely responsible for texture changes and makes a significant contribution to flavor development in ripened cheese. The principal pathway of the proteolytic degradation involves a primary and limited degradation of caseins by rennet and plasmin to polypeptides. These are further degraded to smaller peptides and free amino acids by the proteolytic enzyme system of the starter bacteria (Johnson, 1988; Thomas and Pritchard, 1987; Green and Manning, 1982).

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Figure 1.11 Relationship between extent of acid production up to the draining stage, basic structure of the cheese, and production of flavor (Lawrence et al., 1983).

Factors involving proteolytic breakdown in cheese:

a. Whey separation. The processing step in which the whey is separated from the curd is a key factor in the manufacture of any cheese variety because it determines not only the basic structure of the cheese but also the proportion of residual calf rennet and plasmin in the cheese. Further, their relative activities which are important during cheese ripening are largely influenced by the composition of the cheese curd (Thomas and Pearce, 1981; Lawrence and Gilles, 1986).

b. Rennet. The principal function of rennet is to initiate coagulation of liquid cheese milk to form cheese curd. A proportion of rennet is retained in the curd and plays an important role in the initial stage of proteolytic breakdown of casein in the early stages of cheese ripening. The retention of calf rennet in the curd and its proteolytic activity are pH dependent. The lower the pH of the curd at draining, the greater the rennet retention in the curd and the greater the activity of the rennet. Microbial rennet retention in the cheese curd is not affected by the pH (Holmes et al., 1977).

In the early stages of cheese ripening rennet plays a significant role in the initial breakdown of α_{s1}-casein at the Phe_{23}-Phe_{24} and (or) Phe_{24}-Val_{25} bond to form α_{s1}-I peptide, consisting of residues 24/25 to 199 (De Koning et al., 1981). This results in marked weakening of the casein network and therefore significant change in cheese texture (Creamer and Richardson, 1974; Visser and De Groot-Mostert, 1977). The proteolytic activity of rennet on α_{s1}-casein is stimulated by NaCl concentrations up to an optimum at about 5% but it is inhibited at higher concentrations. However, the proteolysis of α_{s1}-casein can still occur up to 20%

NaCl. In contrast, proteolysis of β-casein is inhibited by 5% NaCl and completely inhibited by 10% NaCl. Therefore, in cheese, β-casein degradation occurs only slowly due to the salt-induced conformational change on β-casein and inacessibility of rennet sensitive bonds (Guinee and Fox, 1987). The sensitive bonds of β-casein are Ala_{189}-Phe_{190} and Leu_{192}-Tyr_{193} (Law and Kolstad, 1983). Other caseins, k- and α_{s2}-casein are resistant to rennet. They contain cystine, existing as disulfide cross links which may be responsible for their insensitivity to rennet attack (Lawrence, 1989).

c. Plasmin, a serine proteinase, is an indigeneous milk alkaline proteinase, associated with casein micelles and dissociated from the micelles as the pH decreases. Its retention and proteolytic activity are also pH dependent. The enzyme can survive pasteurization temperature processes: HTST (72°C, 15 sec) and LTLT (63°C, 30 min). Maximum activity of plasmin is at pH 7.5, 37°C (Grufferty and Fox, 1988). The retention and activity of plasmin is highest in Swiss cheese curd, because the whey is separated from the curd at a relatively high pH, and lowest in Cheshire cheese curd (Lawrence et al., 1983). Plasmin is highly specific for peptide bonds on the C-terminal side of lysine or arginine residues in β- and α_{s2}-casein (Grufferty and Fox, 1988). Activity of plasmin in cheese is stimulated by low concentrations of NaCl up to a maximum of 2% but is inhibited by higher concentrations of NaCl, although some activity remains at 8% NaCl (Guinee and Fox, 1987).

d. The proteinase and peptidase systems of starter bacteria can only slowly degrade whole casein to low molecular weight peptides

(< 1,400) and free amino acids (Visser et al., 1977). Therefore, the main role of starter proteolytic enzymes appears to be the effective degradation of the polypeptides derived from rennet or plasmin action, to small peptides and amino acids which contribute to texture and flavor development in ripened cheese (Thomas and Pritchard, 1987; Green, 1977). This is confirmed by cheese made with rennet alone which contains limited amounts of small peptides and amino acids, while cheese made with rennet and starter contains relatively large amounts of free amino acids (Law, 1984).

Although proteolytic enzymes of starter bacteria play a significant role in further breakdown of polypeptides derived from casein and the development of cheese texture and flavor, they are poorly characterized and there is no information available on the proteolytic cleavage of specific bonds (Law, 1987). Furthermore, it is not known at present for almost all cheese varieties which chemical compounds actually contribute to characteristic cheese flavor (Lawrence and Gilles, 1987; Lawrence et al., 1983).

e. Composition of cheese curd. The final stage in cheese manufacture is to provide the composition of the cheese curd which encourages the microbial and enzymatic activities that produce characteristic cheese texture and flavor (Lawrence and Gilles, 1986; Green et al, 1981a). The important factors are:

- Salt to moisture ratio (S/M)

During cheese ripening proteolytic degradation is markedly influenced by S/M in the cheese. S/M is the major influence on water activity. Therefore, it determines the rate of bacterial growth and proteolytic activities of rennet, plasmin and starter bacteria (Thomas and Pearce, 1981). This is evidenced by a negative relationship between the rate of degradation of both α_{s1}- and β-casein and the level of S/M during cheese ripening. The higher the level of S/M the lower the rate of casein degradation (Gilles and Lawrence, 1973).

- Moisture to casein ratio

Moisture to casein ratio gives a better indication of potential cheese quality than moisture content of the cheese. It is clear that the lower the ratio of moisture to casein, the firmer the casein matrix of the cheese. In addition the moisture to casein ratio indicates the relative hydration of the casein which in turn affects the rate of proteolysis in the cheese. If the moisture to casein ratio is high the rate of casein degradation will be faster, thus the cheese will deteriorate in quality more rapidly after reaching its optimum quality (Lawrence and Gilles, 1980). However, in practice it is difficult for the commercial cheese plant to measure the casein content accurately. Therefore moisture to non-fat substance (MNFS) ratio rather than moisture to casein ratio is commonly used. The non-fat substance is equal to moisture plus solids-not-fat. The solids-not-fat consists of about 4% ash and about 85% casein. Consequently, changes of MNFS correlate closely with changes in the moisture to casein ratio.

- The final cheese pH after salting

The pH is important to give an indication of acid production throughout the cheese manufacturing process. The curd acidity at salting is the most important factor determining the pH of the cheese. The salting pH is largely controlled by the pH at draining. Further decrease in pH after salting depends upon the residual lactose and buffer capacity of the curd. The residual lactose in turn will be influenced by the S/M and the salt tolerance of the starter strains used (Lawrence and Gilles, 1982). The final pH after salting influences the activities of rennet, plasmin and starter enzymes which are important in proteolytic breakdown of casein during cheese ripening (Lawrence and Gilles, 1986).

The manufacture of all cheese varieties involves four major factors: i. the pH of the curd at whey draining, ii. salt to moisture ratio, iii. moisture to casein ratio or MNFS, and iv. the final cheese pH after salting. Each of these four factors can be altered to give specific cheese varieties. Each cheese variety develops a characteristic texture and flavor during maturation, which is largely influenced by the extent of casein degradation. This in turn is influenced by the four major factors (Lawrence and Gilles, 1986). New Zealand research workers (Lawrence and Gilles, 1987; Gilles and Lawrence, 1973) have established the relationship between the composition of Cheddar cheese 14 days after manufacture and quality. This has been successfully and widely used in commercial schemes in New Zealand for assessing Cheddar cheese quality. The relationship is illustrated in Figure 1.12. They suggest a range of S/M (4.7-5.7), MNFS (52-54%), pH (5.1-5.3) and FDM (52-56%) for first grade Cheddar cheese (inner square). For the quality of second grade Cheddar cheese the composition is given in the outer square.

Fat-in-the-dry-matter (FDM)

The FDM is a less important factor than the MNFS, S/M and the pH. However, FDM contributes to flavor development in Cheddar cheese because cheese made from skim milk does not develop a typical flavor. Cheese with FDM greater than 50% develops typical flavor while cheese

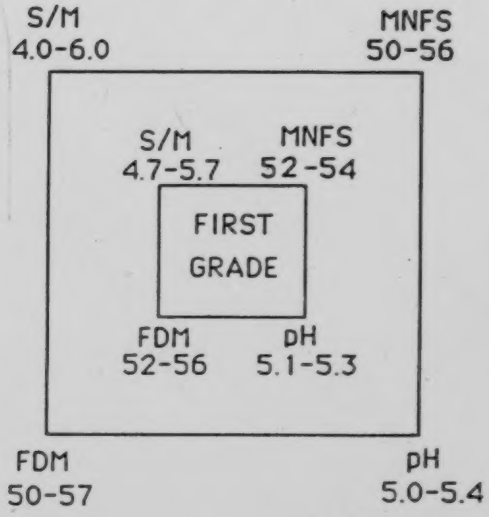
S/M
4.0-6.0
MNFS
50-56
S/M
4.7-5.7
MNFS
52-54
FIRST
GRADE
FDM
52-56
pH
5.1-5.3
FDM
50-57
pH
5.0-5.4

Figure 1.12 Suggested ranges of salt-in-moisture (S/M), moisture in non-fat substance (MNFS), fat-in-the-dry matter (FDM) and pH for First grade (inner square) and Second grade Cheddar cheese. Analyses 14 days after manufacture (Lawrence and Gilles, 1987).

with FDM less than 50% does not (Lawrence and Gilles, 1987). Lipolytic breakdown of milk fat probably occurs in all cheese varieties. However, the rate and extent of lipolysis varies considerably between cheese varieties. Although it is clear that milk protein and lactose are the important sources of flavor precusors in Cheddar cheese, milk fat also plays an important but not yet completely defined role. The contribution of fat and its breakdown products to flavor in Cheddar cheese is still a matter of debate (Johnson, 1988; Lawrence and Gilles, 1987).

ii. Glycolytic degradation of lactose

Glycolysis of lactose to lactic acid by starter bacteria in cheeses is essential in determining cheese pH during cheese manufacturing and early stages of ripening. This influences mineral retention and therefore basic structure of the cheese. It also controls proteolytic degradation of casein during ripening which is essential for characteristic cheese texture and flavor (see section 1.6.3i). Lactic acid contributes to the sharp acid flavor in unripened cheese such as Cottage cheese (Law, 1984). Furthermore, the lactic acid inhibits the growth of undesirable microorganisms which may cuase spoilage and food poisoning (Kosikowski, 1986).

iii. Lipolytic degradation of milk fat

Lipolysis is important in mold ripened cheeses and several Italian type cheeses. Important sources of lipases are milk, microorganisms (e.g. *Penicillium* spp.) and rennet paste (which contains lipolytic enzymes in addition to rennet) (Wong, 1974). The action of these lipolytic enzymes on milk fat liberates free fatty acids, such as butyric, caproic, caprylic and capric acids. These low molecular fatty acids are then converted to methyl ketones by β-oxidation and decarboxylation which are important for flavor of mold ripened cheeses (Johnson, 1988; Hawke, 1966).

In Italian type cheeses, free fatty acids produced from the lipolysis of milk fat are essential for the cheese flavor such as Romano cheese. Butyric acid is related to the characteristic flavor intensity of the cheese (Wong, 1974).

In conclusion, scientific information of cheese manufacturing and ripening from normal milk is very well established. However, UF milk can be considered as a new raw material because of marked changes in its chemical composition and physical properties. Therefore there is a need for more research on the physical, chemical, biological and processing properties of UF milk. The factors involved to control these properties must be understood for the successful application of UF milk as a raw material for the manufacture of cheese varieties and other dairy and food products.

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2. THE EFFECT OF ULTRAFILTRATION ON PHYSICOCHEMICAL PROPERTIES OF SKIM MILK RETENTATE[1]

2.1 Introduction

Ultrafiltration (UF) is a membrane process used for fractionation, clarification, and selective concentration of emulsified, colloidal and dissolved materials in an aqueous system. Its function is based upon a sieving process. High molecular weight components are separated and concentrated from low molecular weight components at low temperature and low pressure, thus less energy is required when compared with the energy needs of conventional evaporation processes. Under mild UF conditions (no extreme physical or chemical conditions are used), there are little or no detrimental changes in the nutritional, functional (protein denaturation) and sensory qualities of food products (Lewis, 1982; Hedrick, 1983/1984; Kosikowski, 1986a).

In dairy technology the UF process has undergone many advances and is now being employed commercially. Milk products concentrated by UF include whole milk, skim milk and buttermilk (Kosikowski, 1986b).

Application of UF for cheesemaking was introduced by Maubois, Mocquot and Vassal (1969). The principal concept is based upon concentration of milk to the level where its composition is similar to the desired cheese. This concentrate is called "precheese", to which the rennet and starter cultures are added. Thus, the cheese is produced with little or no whey loss (Maubois and Mocquot, 1975). Ultrafiltered milk has been used successfully for the manufacture of soft and semi-soft cheese (Covacevich and Kosikowski, 1978; Fox, 1984) and its application has increased significantly in the cheese industry, due to several economic and technical advantages. For example, ultrafiltration of milk on the farm resulted in a reduced milk volume, thus savings in refrigeration and transportation costs from the farm (Jameson, 1983), and also in processing cost (heating and cooling) and manufacturing time at the dairy (Patel and Reuter, 1985). Incorporation of whey proteins in the cheese results in a distinctive increase in nutritional value and cheese yield (Yan *et al.*, 1979; Ernstrom *et al.*, 1980; Fernandez and Kosikowski, 1986; Kosikowski, 1986a), and approximately 75% less rennet is required (Kosikowski, 1986b). Other advantages include a high potential for continuous operation in a closed system because cheese vats are not essential and, as a result, a highly sanitary environment can be maintained (Bundgaard *et al.*, 1972; Kosikowski, 1986a). There is also a better control of cheese weight (Kosikowski, 1986b) and a decreased B.O.D. (biological oxygen demand) in waste from a UF cheese plant due to little or no whey loss (Bundgaard *et al.*, 1972; Maubois and Mocquot, 1975).

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[1] A version of this chapter has been published. S. Srilaorkul, L. Ozimek, F. Wolfe and J. Dziuba. 1989. Can. Inst. Food Sci. Technol. J. 22(1):56.

However, some major problems still exist in the use of UF milk for cheesemaking, particularly for the manufacturing of hard or processed cheeses. These problems include an increase in buffer capacity of the precheese and changes in physicochemical properties. The strong buffer capacity causes difficulties in obtaining optimal pH for cheese quality and in an inability to reduce pH and repress growth of spoilage or pathogenic microorganisms (Kosikowski, 1986a). Additionally, the cheese produced can be poor in body, texture and flavor (Glover, 1985). These defects are critical since body, texture and flavor are important criteria for the cheese quality (Lawrence *et al.*, 1983).

The objective of this study was to examine the effect of skim milk concentration by ultrafiltration on chemical composition, buffer capacity and buffer intensity system of retentate.

2.2 Materials and Methods

2.2.1 Preparation of Retentate

A laboratory DDS-20 unit with twenty 0.018 m^2 membranes with molecular weight cut-off of 25,000 daltons was used for separation and concentration. Commercial HTST pasteurized skim milk was ultrafiltered at 50 $\pm$ 1^{o}C, with inlet and outlet pressures of 400 and 320 kPa, respectively. The extent of the ultrafiltration process was measured by the volumetric concentration factor (VCF) where:

$$\text{VCF} = \frac{\text{Volume of original feed}}{\text{Volume of final concentrate}} \qquad (2.1)$$

Part of the skim milk was kept and used as a control which was expressed as VCF 1:1.

2.2.2 Analytical Methods

The samples of skim milk, retentate and permeate were analyzed for dry matter, protein, fat, lactose, ash, Ca, P, Mg, Na and K. Dry matter was determined by the hot air oven method (AOAC, 1984). Total protein, casein, whey proteins and non-protein nitrogen were determined by the macro-Kjeldahl method (AOAC, 1984). The samples of retentate were diluted with water to the original milk volume before analyses were performed. Fat was determined by the Mojonnier procedure (Atherton and Newlander, 1977). Lactose was determined by the sulfuric acid and phenol colorimetric method (Lawrence, 1968). Ash was determined by drying at 100°C and then igniting in a muffle furnace at 550°C. For mineral content, P was determined by the phosphomolybdate colorimetric method after ashing (Australian Standard, 1974), Ca and Mg by atomic absorption spectrophotometry, and K and Na by flame emission spectrophotometry using a Perkin-Elmer 4000 Spectrophotometer (Perkin-Elmer Corporation, Norwalk, CT).

2.2.3 Measurement of Buffer Capacity

Twenty milliliters of skim milk or retentate at 20°C was titrated with 0.1 N sodium hydroxide at 15 min intervals until pH 9 was obtained. Similarly, to 20 mL of retentate, 0.1 N hydrochloric acid was added and the pH was measured until pH 4.4 was reached. Titration curves were drawn, plotting the amount of 0.1 N NaOH and 0.1 N HCl *vs* pH. The buffer index (dB/dpH) was calculated by using the following equation:

$$dB/dpH = (\text{mL acid or base})(\text{normality factor})/(\text{volume of sample})(\Delta pH) \qquad (2.2)$$

Buffer capacity curves were obtained by plotting the buffer index against pH.

2.2.4 Determination of Buffer System Intensity

The buffer system intensity of skim milk and retentate components was determined by a slight modification of the method of Kirchmeier (1980). The skim milk or retentate (100 mL) was acidified with 1N HCl to pH 4.6 to precipitate and remove casein. Whey proteins and soluble solids (milk salts, lactose and non-protein nitrogen) were separated by an ultrafiltration unit having a flat sheet membrane with molecular weight cut-off of 10,000 daltons. The casein, whey proteins, milk salts and other soluble solids were freeze-dried. Before analysis each freeze-dried portion was brought up to 100 mL with deionized water. Then, 20 mL of the solution or dispersion was titrated with 0.1 N NaOH until pH 7.0 was obtained.

2.2.5 Statistical Methods

Results were expressed as means of three replicates or as otherwise indicated. Data were analyzed using analysis of variance and Duncan's multiple range test. Correlations were determined by simple linear regression.

2.3 Results and Discussion

Composition of Retentate

Chemical composition of skim milk and retentate of different volumetric concentration factors are given in Table 2.1. The content of casein, whey proteins, lactose, non-protein nitrogen and minerals in skim milk was in a typical range observed in normal skim milk. During concentration of skim milk by ultrafiltration, the concentration of casein and whey proteins increased in proportion to the concentration factor (VCF). In practice, the measurement of protein content in retentate could be accurately used to determine the concentration factor of the UF retentate. The lactose concentration decreased as the VCF increased. However, the percent of lactose removed from skim milk increased to 84-89% during ultrafiltration to volume concentration factor 5:1 (Table 2.1).

There was a linear increase in mineral concentration (Ca, P, Mg, K and Na) as the concentration factor increased (Figure 2.1), which is reflected by the increase in ash concentration (Table 2.1). However, the increase in mineral concentration was less extensive than that of milk proteins. Thus, the concentration of individual minerals, expressed in mg/g of casein, decreased significantly ($P < 0.05$) to VCF 3 except for Ca which decreased significantly ($P < 0.05$) to VCF 2 (Table 2.2). Further concentration of milk up to VCF 5 did not affect the proportion of Ca, P, Mg and casein. This indicates that soluble Ca was removed from milk during two-fold concentration, while a three-fold concentration of the milk was required for removal of soluble P and Mg. In the retentate, the remaining colloidal form of

Table 2.1 The effect of concentration of skim milk by ultrafiltration on the chemical composition of retentate and permeate.

VCF^1	DM^2 (%)^3	Casein (%)^3	Whey proteins (%)^3	NPN (%)^3	Fat (%)^3	Lactose (%)^3	Ash (%)^3
Retentate							
1	8.36±0.28	2.42±0.07	0.76±0.16	0.17±0.01	0.15±.06	4.90±0.10	0.77±0.01
2	12.54±0.34	4.90±0.82	1.65±0.11	0.17±0.02	0.28±.08	4.90±0.07	1.06±0.03
3	15.36±0.39	7.40±0.34	1.80±0.62	0.20±0.01	0.54±.06	4.82±0.09	1.28±0.02
4	18.98±0.11	9.64±0.58	2.65±0.16	0.22±0.06	0.82±.05	4.77±0.10	1.58±0.04
5	22.78±0.12	12.05±0.35	3.60±0.28	0.26±0.06	1.20±0.12	4.54±0.13	1.79±0.05
Permeate							
2	5.13(30.7)^4	nd^5	nd	0.17(50.0)^4	nd	4.80(43.0)^4	0.47(30.5)^4
3	5.17(41.2)	nd	nd	0.17(66.6)	nd	5.11(78.2)	0.48(46.8)
4	5.32(46.4)	nd	nd	0.17(75.0)	nd	5.11(78.2)	0.48(46.8)
5	5.40(51.7)	nd	nd	0.17(80.0)	nd	5.20(84.9)	0.48(49.9)

[1] VCF - Volume concentration factor [2] DM - Dry matter [3] Mean ± SD

[4] Percent of dry matter (DM), NPN, lactose and ash removed from skim milk, respectively.

[5] nd - Not detectable

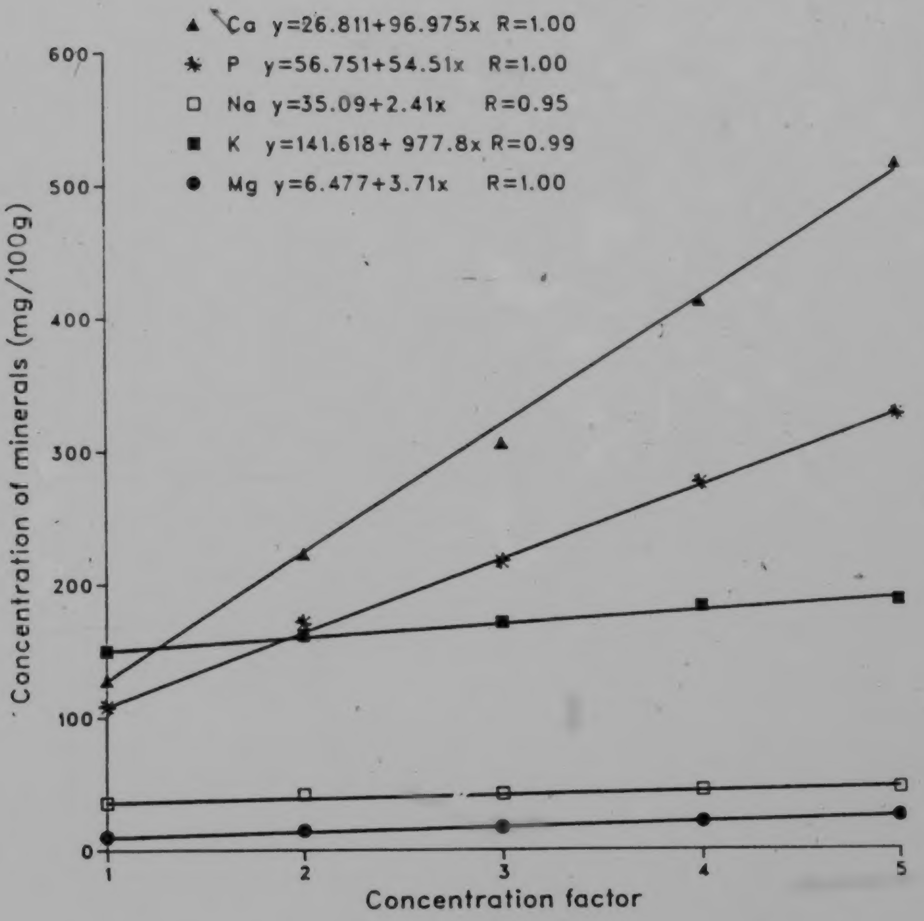

Fig. 2.1. The content of minerals in skim milk and retentate.

Table 2.2. The effect of UF concentration of skim milk on content of some mineral constituents.

Volume concentration factor (VCF)	Minerals content (mg/g casein)^{1}				
	Ca	P	Mg	Na	K
1	53.0 ± 2.79^{a}	44.9 ± 4.88^{a}	4.0 ± 0.14^{a}	14.8 ± 1.87^{a}	62.0 ± 1.17^{a}
2	45.5 ± 0.06^{b}	34.9 ± 9.48^{b}	3.0 ± 0.07^{b}	8.5 ± 0.79^{b}	33.0 ± 1.03^{b}
3	41.3 ± 1.61^{b}	29.3 ± 0.27^{c}	2.3 ± 0.16^{c}	5.7 ± 1.01^{c}	23.1 ± 1.55^{c}
4	42.9 ± 1.52^{b}	28.7 ± 0.40^{c}	2.2 ± 0.08^{c}	4.7 ± 0.98^{c}	$19.0\pm1.69^{c,d}$
5	43.0 ± 1.75^{b}	27.3 ± 0.84^{c}	2.1 ± 0.12^{c}	3.9 ± 0.57^{c}	15.6 ± 0.56^{d}

[1] Mean ± SD

^{a,b,c,d}Different letters in the column indicate significant differences $P<0.05$

minerals was in a casein-calcium (magnesium)-phosphate complex. Further reduction of the mineral content in the colloidal form could be carried out by decreasing the pH of milk and diafiltration process (Quist et al., 1987).

Composition of Permeate

During concentration of skim milk by ultrafiltration, using membranes with molecular weight cut-off of 25,000 daltons, neither protein nor fat was detected in the permeate (Table 2.1). This was due to the fact that these components have higher molecular weights and were completely retained by the membrane. The concentration of non-protein nitrogen and ash remained fairly constant regardless of the change of the concentration factor. There was no significant change in concentration of Ca, P, Mg, Na ($P > 0.05$) and K ($P > 0.01$) as concentration factor increased (Figure 2.2) which corresponded to the ash content of permeate.

Buffer Capacity of Retentate

The buffer capacity curves of skim milk and retentate are shown in Figure 2.3. During the course of ultrafiltration, the buffer capacity increased steadily. This was due to increased casein, whey proteins and milk salts in the retentate. Maximum buffer capacity of skim milk was observed at pH 5.4. At this pH of milk there was hydrolytic removal of colloidal phosphate from casein (Walstra and Jenness, 1984; Kirchmeier, 1980), according to the following equation:

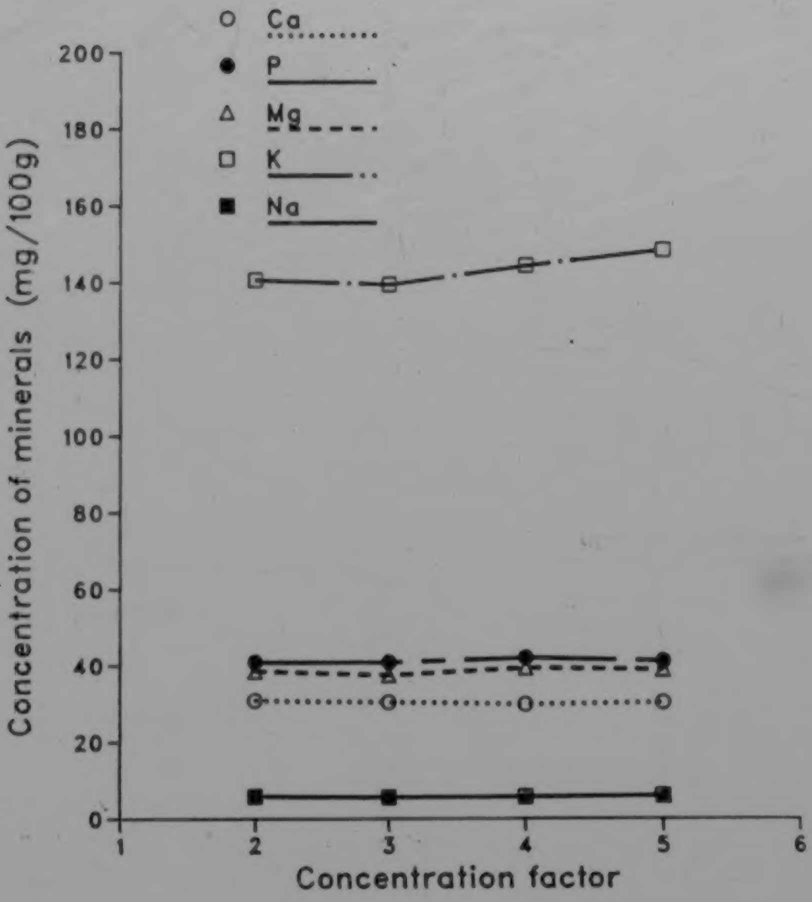

Fig. 2.2. The content of minerals in permeate.

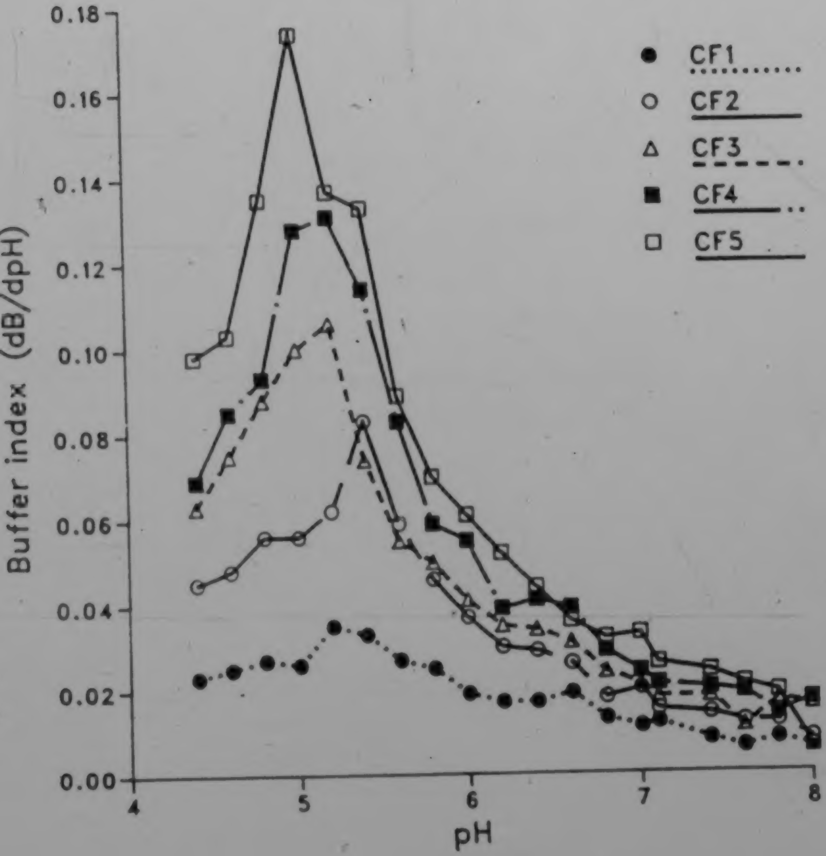

Fig. 2.3. Buffer capacity curves of skim milk and retentate.

$$\text{Calcium-casein-(magnesium)-phosphate complex} \rightleftharpoons \text{H -casein} + \text{soluble calcium-(magnesium)-phosphate} \qquad (2.3)$$

The maximum buffer capacity increased in direct proportion to the degree of concentration of retentate (Figure 2.4). However, it was observed that the maximum buffer capacity was moving towards a lower pH as the concentration factor increased (Figure 2.3). In retentate of VCF 5 the maximum buffer capacity was observed at pH 5. The shift in the maximum buffer capacity from pH 5.4 to pH 5.0 might be caused by the increased concentration of milk proteins. It should be mentioned that the isoelectric point of whey proteins is pH 5.0-5.2. Dziuba and Bochenek (1984) reported that buffer capacity of native whey proteins was two-fold higher at pH 5.0-5.2 than at pH of normal milk (pH 6.6-6.8).

Buffer System Intensity of Retentate Components

The buffer system intensity of individual components of retentate was determined by measurement of the amount of NaOH used during titration of retentate in the range of pH from 4.6 to 7.0. The results obtained are presented in Table 2.3 and Figure 2.5. The contribution of casein, whey proteins and milk salts to the total buffer system intensity of skim milk from this study was 36.0%, 5.4% and 58.6%, respectively. The results obtained were comparable to the strength of the buffer system of milk (casein 26.3%, whey proteins - 8.3% and milk salts - 65.1%) reported by Kirchmeier (1980). As the concentration

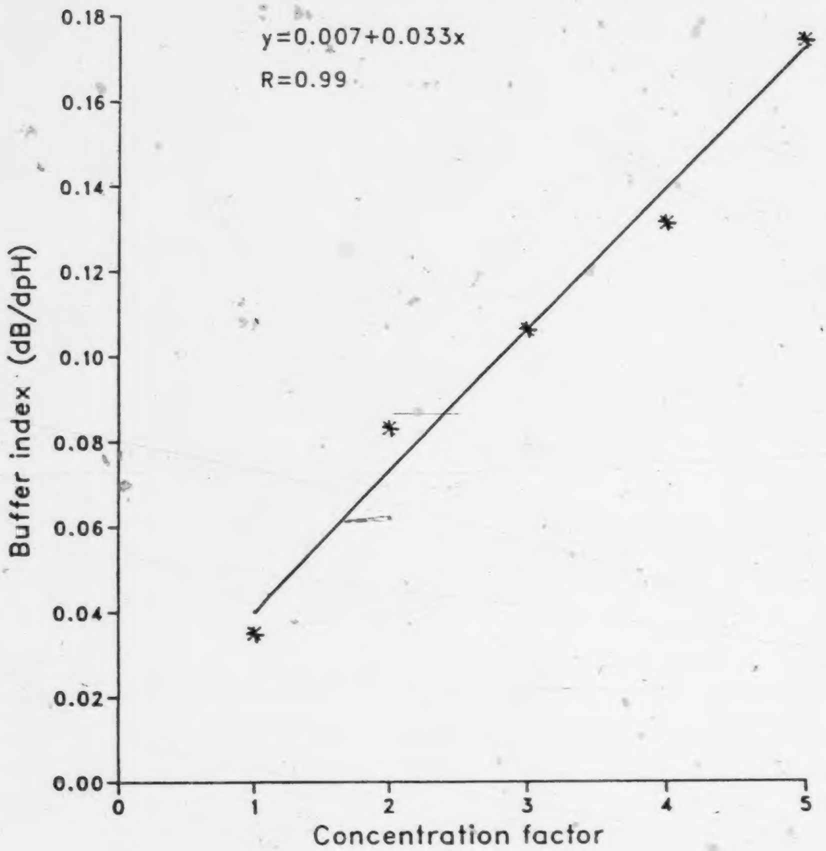

Fig. 2.4. The effect of UF concentration of skim milk on its maximum buffer capacity.

Table 2.3. The buffer system intensity of major components of skim milk and retentate (titration range pH 4.6-7.0).

	Relative Intensity (%)[1]				
	Volume Concentration Factor (VCF)				
Buffer System	1	2	3	4	5
Whey proteins	5.4	6.1	7.5	6.9	9.7
Casein	36.0	47.2	48.5	52.3	53.8
Milk salts	58.6	46.7	44.0	40.8	36.5

[1] Mean of 2 replicates.

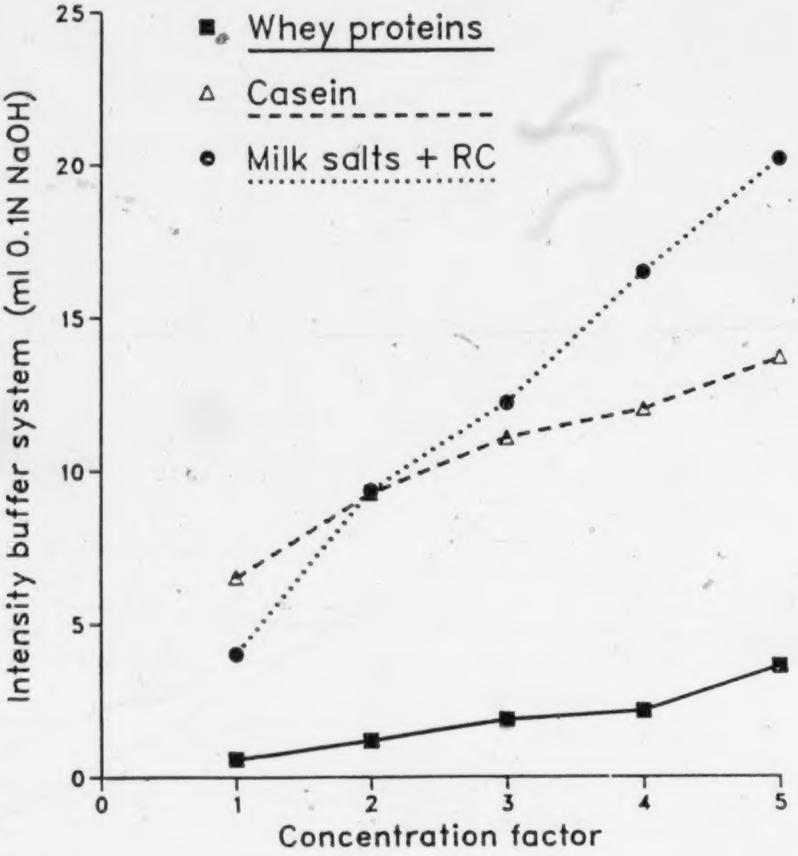

Fig. 2.5. Effect of UF concentration of skim milk on buffer system intensity; titration range pH 4.6-7.0

factor increased, the contribution of proteins to the total buffer system intensity increased but that of milk salts decreased. This is related to the relatively higher increase in milk protein concentration when compared with that of milk salts during ultrafiltration. The contribution of casein, whey proteins and milk salts to the total system intensity of five-fold retentate was 53.8%, 9.7% and 36.5%, respectively. Changes in the total buffer system intensity (BSI) of dry matter components of retentate in relation to the concentration factor showed a positive linear relationship as presented in Figure 2.6. Similar positive linear relationships were found between casein content and its BSI (Figure 2.7), whey proteins and its BSI (Figure 2.8), and milk salts concentration and its BSI (Figure 2.9).

Changes in the physicochemical properties of UF milk retentate such as chemical composition and buffer capacity could substantially affect the nutritional, safety (Kosikowski, 1986a) and sensory quality (Glover, 1985) aspects of the final products. In particular, it could affect technological processes where ultrafiltered milk is used. For example, the use of ultrafiltered milk for soft cheesemaking could eliminate the draining step from the curd (Maubois and Mocquot, 1975). Intermediate concentrations of retentates can also be used, resulting in reduced syneresis of the curd. However, a significant increase in buffer capacity of retentate affects the growth and activity of starter cultures. To obtain a desired pH of retentate, the starter culture must produce much more lactic acid than it would if grown in milk to overcome the buffer capacity effect. An alternative is the direct addition of acid to the retentate. However, it was found that the amount of lactic acid (weak acid) required to change the pH by a unit

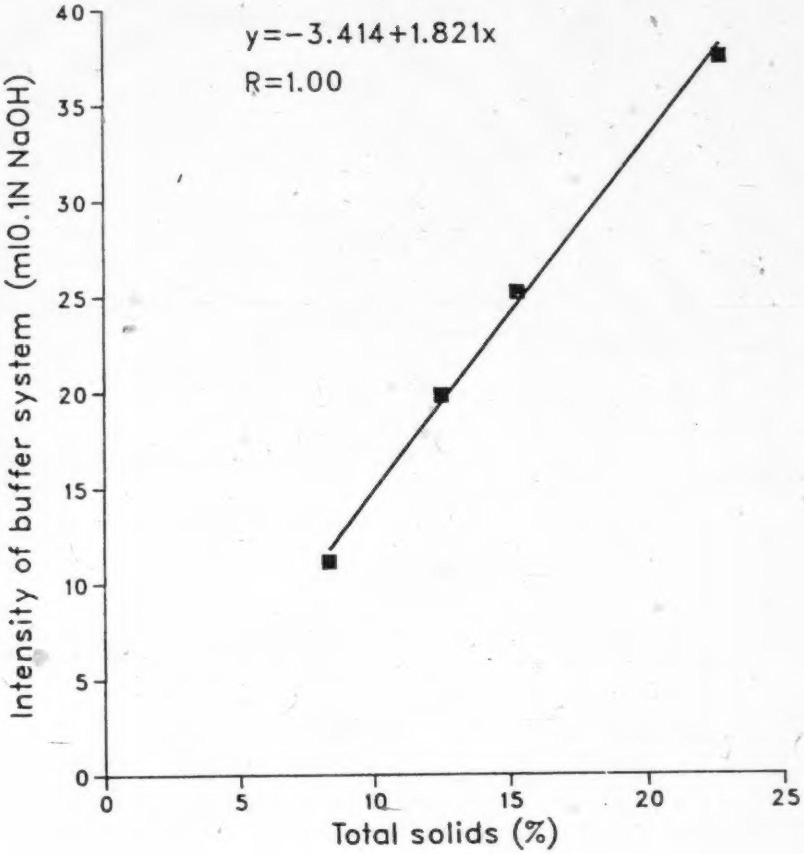

Fig. 2.6. The effect of total solids content in retentate on buffer system intensity; titration range pH 4.6-7.0

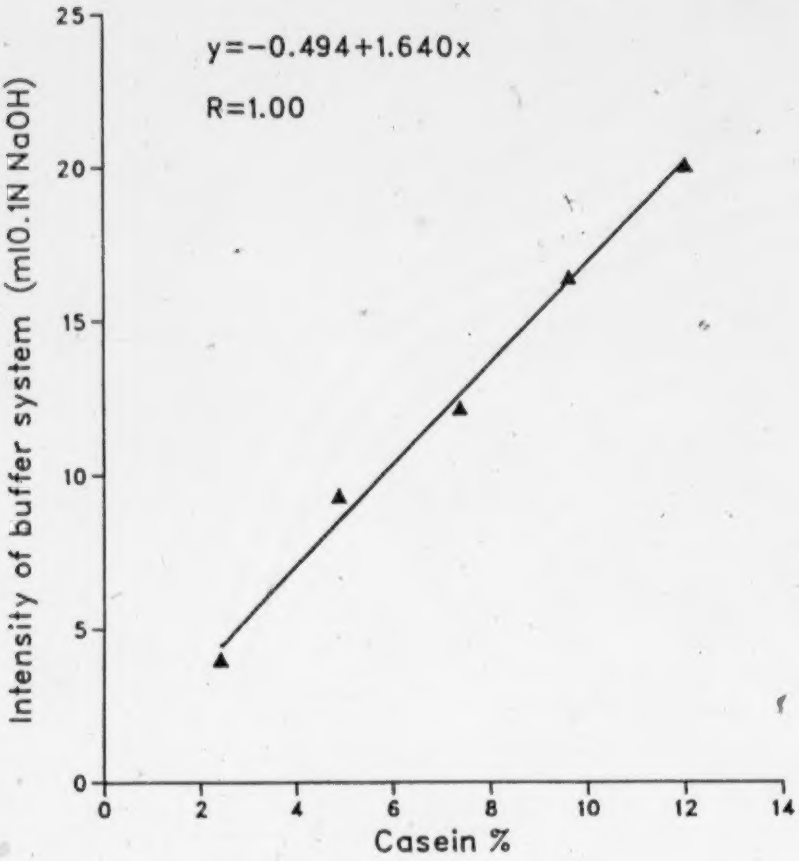

Fig. 2.7. The effect of casein content in retentate on buffer system intensity; titration range pH 4.6-7.0

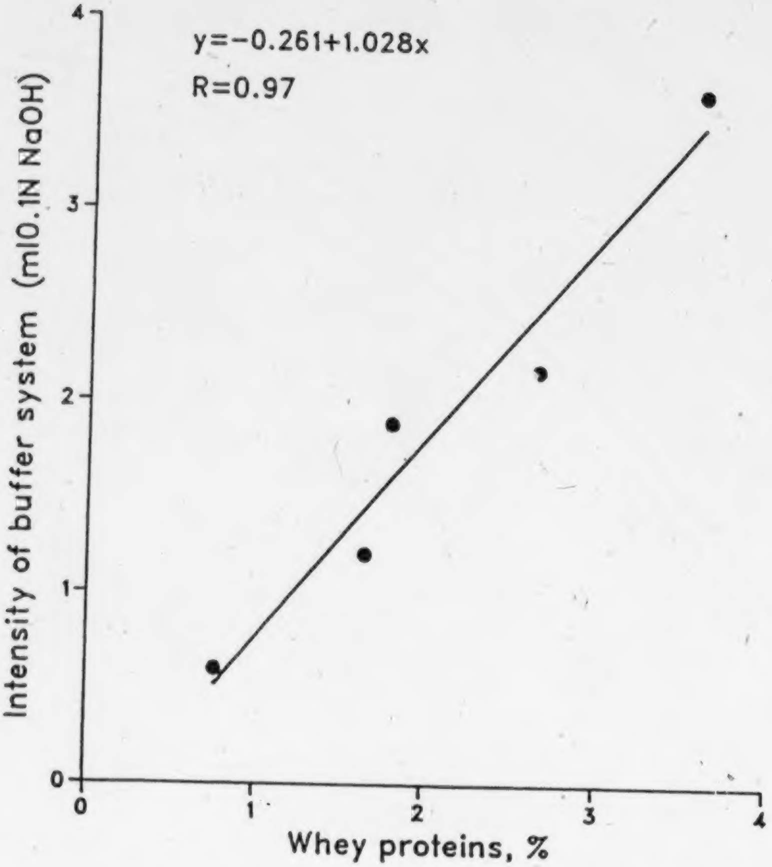

Fig. 2.8. The effect of whey protein content in retentate on buffer system intensity; titration range pH 4.6-7.0

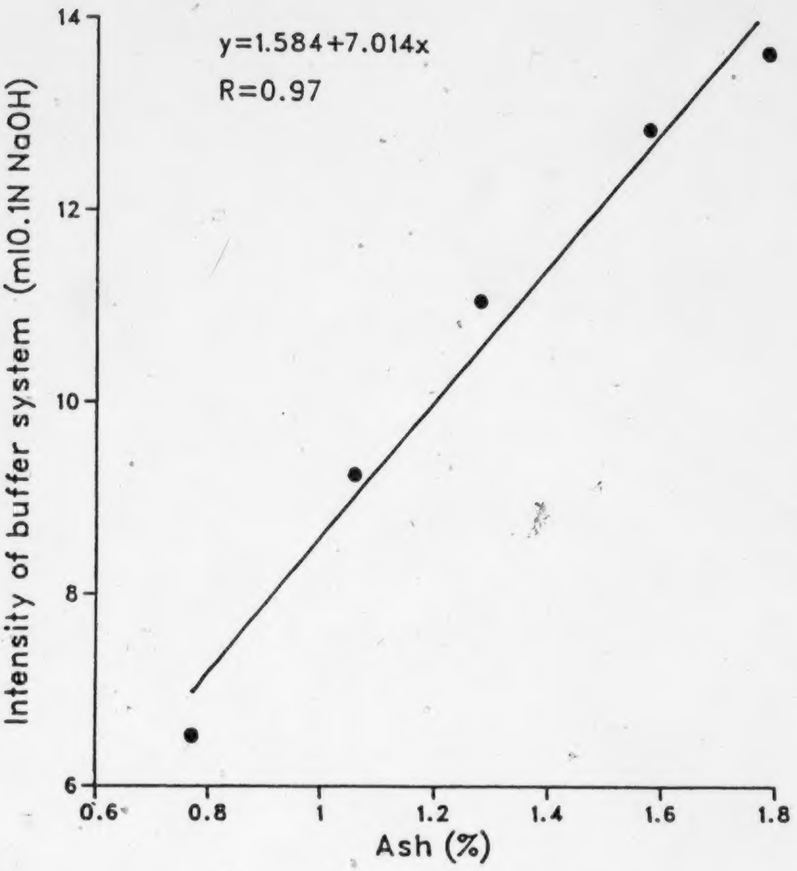

Fig. 2.9. The effect of milk salt content in retentate on buffer system intensity; titration range pH 4.6-7.0

was much greater than that of HCl (strong acid) and was dependent on the retentate concentration factor (Figure 2.10a,b).

On the other hand, the higher buffer capacity of retentate might have a positive effect on the quality of some fermented milk products (e.g. yogurt) which are adversely affected by changes in pH during distribution and storage.

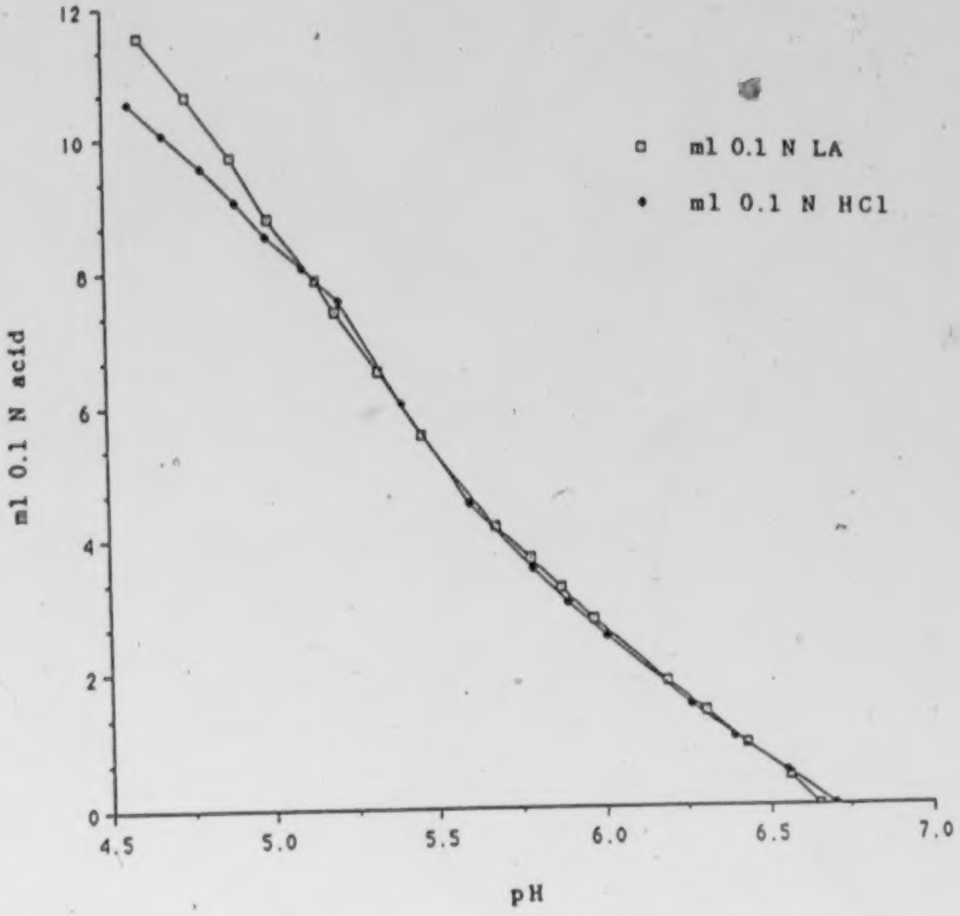

Fig. 2.10a. The effect of lactic acid and hydrochloric acid on titration curve of skim milk.

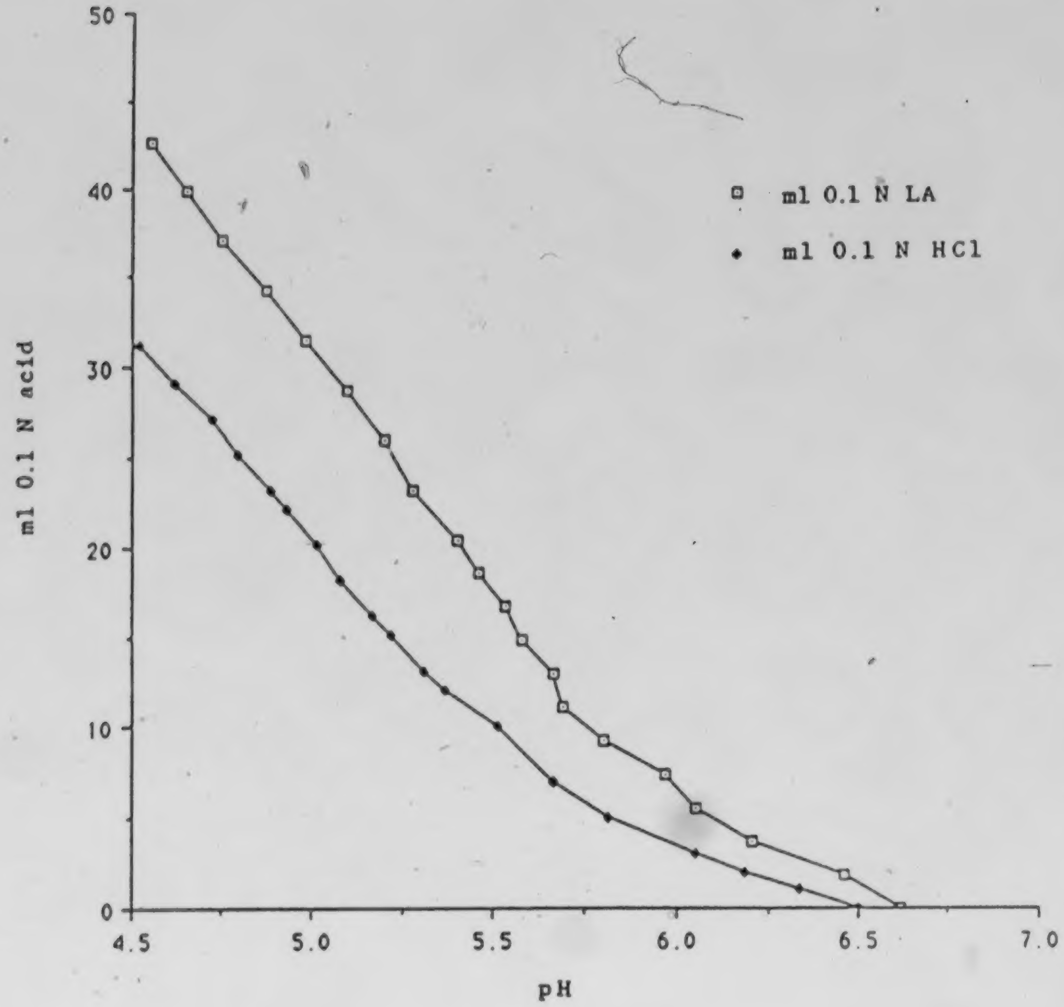

Fig. 2.10b. The effect of lactic acid and hydrochloric acid on titration curve of 4-fold ultrafiltered skim milk concentrate.

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3. GROWTH AND ACTIVITY OF *Lactococcus lactis* subsp. *cremoris* IN ULTRAFILTERED SKIM MILK[1]

3.1 Introduction

Interest in use of ultrafiltration (UF) of milk for cheesemaking is mainly due to the major increase in cheese yield by incorporation of whey proteins into the cheese. Other advantages include savings in energy, rennet, starter culture, manufacturing time and space and whey disposal (8, 9, 17, 24). However, the ultimate goals of cheesemaking are not only high production efficiency and maximum yield but also quality characteristics such as flavor and texture. There are many factors which influence cheese quality. A major factor is the starter culture which plays many critical roles in the development of cheese quality (32, 33). These include a) production of lactic acid which gives fresh acid flavor to the cheese curd and assists in milk coagulation, b) shrinkage of the curd and moisture expulsion thus promoting characteristic texture formation during cheesemaking, c) inhibition of growth of microbial spoilage bacteria and pathogens and, d) production of flavorful compounds.

The ability of starter cultures to grow and produce sufficient lactic acid and enzymes for conversion of milk to cheese depends on environmental factors such as temperature, pH and composition of the milk (19). UF is a membrane separation and concentration process, in which macromolecules and solutes of specific shape and molecular weight are retained while low molecular weight constituents pass through the membrane and are separated from the UF milk retentate. Thus UF of milk causes a change in the composition of the milk which could affect the growth and activity of the starter cultures and consequently cheese quality. Furthermore the high buffer capacity of UF milk retentate makes it more difficult to achieve the desired low pH which controls cheese quality (texture and flavor) as well as growth of microbial spoilage organisms and pathogenic bacteria (18).

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[1] A version of this chapter has been accepted for publication. S. Srilaorkul, L. Ozimek and M.E. Stiles. 1989. J. Dairy Sci. (In press).

Although starter cultures play a critical role in development of good quality cheese, there is limited information available on their performance in UF milk retentates. The objectives of this study were to determine the effect of UF concentration of skim milk on growth, lactic acid production and proteolysis of *Lactococcus lactis* subsp. *cremoris* used in cheesemaking. In addition, the amount of starter culture required to achieve desired pH change in cheesemaking from UF milk retentate was determined.

3.2 Materials and Methods

3.2.1 Preparation of Milk Retentate

A laboratory ultrafiltration unit (DDS-20 LAB-module, Nakskov, Denmark) with 0.018 m^2 membrane (GR 60-PP) with molecular weight cut-off 25,000 daltons was used for concentration of the milk. Commercial HTST pasteurized skim milk was ultrafiltered at 50 ± 1°C with inlet and outlet pressures of 400 and 320 kPa, respectively. The milk was concentrated to the following volume concentration factors

(CF) 2:1, 3:1, 4:1 and 5:1, where CF is the volume of original feed/final volume of retentate. Part of the skim milk was kept and used as a control (CF 1:1).

3.2.2 Analytical Methods

Dry matter (DM) was determined by hot air oven method (2); total protein by macro Kjeldahl method; non protein nitrogen (NPN) was determined by the procedure of Rowland (31); casein was determined according to AOAC method (2) using 6.38 as the factor to convert nitrogen to protein content. Whey proteins were calculated by difference between total protein and the sum of casein and NPN (14); fat was determined by the Mojonnier procedure (3); lactose by sulfuric acid and phenol colorimetric method (22); and ash by igniting the dried sample in a muffle furnace at 550°C. Mineral content was determined as follows: phosphorus by the phosphomolybdate colorimetric method after ashing (4), calcium and magnesium by atomic absorption spectrophotometry, and potassium and sodium by flame emission spectrophotometry using a Perkin-Elmer 4000 Spectrophotometer (Perkin-Elmer Corporation, Norwalk, CT).

3.2.3 Measurement of Buffer Capacity

A 20 mL aliquot of retentate at 20°C was titrated with 0.1 N NaOH at 15 min intervals to pH 9.0. Similarly, 20 mL of retentate was titrated with 0.1 N HCl to pH 4.4. Titration curves were drawn, plotting the amounts of 0.1 N NaOH and 0.1 N HCl against pH. Buffer indices (dB/dpH) were calculated according to the method of Van Slyke (38). Buffer capacity curves were obtained by plotting these indices against pH.

Starter Cultures

commercial strains of *Lactococcus lactis* subsp. *cremoris* strains 103, 108 and 208 were obtained from local cheese plants in Alberta. The starter cultures were obtained in frozen form, checked for purity, frozen as stock cultures by freeze preservation in preserving medium containing glycerol and 5% trisodium citrate and stored at -70°C. When required, the frozen cultures were reconstituted by subculturing twice in Elliker Broth (Difco Laboratories Inc., Detroit, MI) at 32°C for 24 h. A 1% inoculum from the Elliker Broth was transferred to an internally pH controlled starter medium (Nordica SS-003, Nordica International, Sioux Falls, S. Dakota), incubated for 16 h at 32°C and used to inoculate the UF retentates.

3.2.4 Starter Activity Test

The activity test developed by Mistry and Kosikowski (25) was used. Two hundred mL of each of the retentates was pasteurized at 63°C for 30 min, cooled to 32°C, inoculated with single strain starter cultures and incubated at 32°C. Size of inoculum used was directly proportional to the protein content of retentate, hence the starter inoculum levels for CF 1 to CF 5 were 1.5, 3.2, 4.4, 5.6 and 7.2%, respectively. Titratable acidity (TA), pH and bacterial count were determined every 2 h until pH 4.6 was attained or for a maximum of 10 h incubation. TA was determined with 0.1 N NaOH and phenolphthalein indicator and reported as percent developed lactic acid (difference between total and original TA). The viable colony forming units

(CFU)/mL was determined on Elliker agar (Difco), incubated anaerobically at 32°C for 48 h. Doubling time for starter cultures and for lactic acid production in the exponential growth phase was calculated by the following formulae (25, 30).

T_{dg} = $0.301\ (t - t_o)/(\log N - \log N_o)$

T_{da} = $0.301\ (t - t_o)/\Delta LA$

where T_{dg} = doubling time for growth

N = colony forming units at time t

N_o = colony forming units at time t_o

T_{da} = doubling time for lactic acid production

ΔLA = difference in percent lactic acid production between time t and t_o

3.2.5 Proteolytic Activity Test

Proteolytic activity of the starter cultures was determined by a modification of the Hull method (6). After incubation for 6 h at 32°C, 5 mL of the above milk retentate was mixed with 1 mL distilled water and 10 mL 0.72 N trichloroacetic acid (TCA). After standing for 10 min the sample was filtered through Whatman filter paper No. 42 and 5 mL of the filtrate was added with 10 mL sodium carbonate reagent and mixed thoroughly before adding 3 mL phenol reagent. The liberated tyrosine and tryptophan of the TCA filtrate was measured at 650 nm by Spectronic 21 (Bausch and Lomb, Rochester, NY). The amount of proteolysis was determined by the difference between the total and original values, and expressed as μg of tyrosine released per ml of milk retentate.

3.2.6 Determination of Inoculum Size

To determine the inoculum size required for the desired change in pH (ΔpH) for Cheddar cheesemaking from UF retentate, 10 mL of pasteurized retentate was inoculated with 0.5, 1, 3, 5 and 10% v/v of starter culture. The activity test was done at 37°C to simulate practical conditions in Cheddar cheesemaking. Initial and final pH after incubation at 37°C for 5 h was used to determine ΔpH (27).

3.3 Results and Discussion

Composition of retentates produced by UF of skim milk to different concentration factors (CF) is given in Table 3.1. There is a linear increase in dry matter (DM) with increased CF. Macromolecules like casein, whey proteins and fat increase with increasing CF because these components are completely retained by the membrane, as reported by Glover (9). Non-protein nitrogen (NPN), which is composed of free amino acids, low molecular weight peptides, urea and uric acid, remains constant regardless of the change of CF. There is a slight decrease in lactose content with increasing CF, but as CF increases the mineral content (Ca, P, Mg, K, and Na) also increases, corresponding to the increased ash content.

The effect of UF skim milk on the growth of, and lactic acid production by *L. lactis* subsp.*cremoris* strains 103, 108 and 208 was determined. Typical growth and lactic acid production are shown in Figures 3.1 and 3.2, respectively. During logarithmic growth of the starter cultures, amount of lactic acid produced (ΔLA) increased as the CF of the retentate increased. After incubation for 6 h at 32°C,

Table 3.1. Composition[a] of Skim Milk and UF Skim Milk Retentates

CF	DM	Casein	Whey Proteins	Fat	NPN	Lactose	Ash	Mineral Content				
								Ca	P	Mg	K	Na
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(mg/100g)				
1	8.82	2.55	0.54	0.06	0.16	5.03	0.76	125.1	96.9	10.7	162.3	44.5
2	13.08	5.20	1.49	0.15	0.16	4.98	1.11	237.0	163.3	15.1	179.2	50.0
3	16.02	7.34	1.83	0.44	0.13	4.89	1.32	319.9	212.5	18.6	185.6	52.3
4	19.11	9.13	2.43	0.88	0.14	4.87	1.51	411.3	256.9	21.1	195.3	55.6
5	22.92	12.62	2.52	1.03	0.15	4.73	1.88	511.3	329.5	24.4	206.8	59.4

[a] Means of three replicates.

CF - Concentration factor

DM - Dry matter

NPN - Non-protein nitrogen

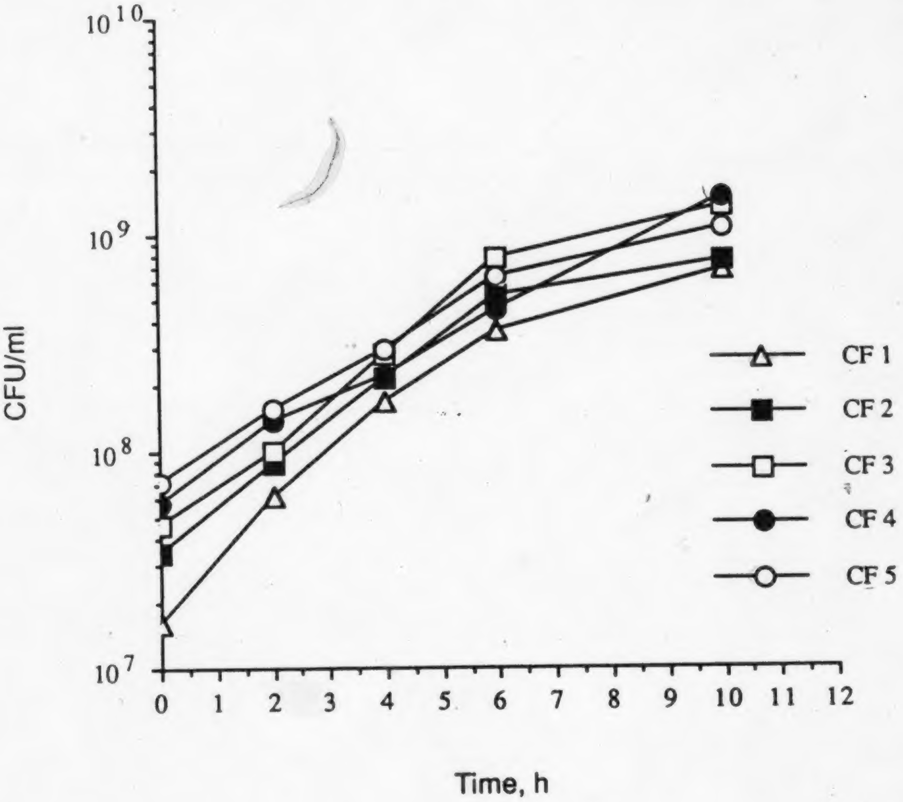

Figure 3.1. Growth of *L. lactis* subsp. *cremoris* strain 103 in ultrafiltered skim milk of different concentration factors (CF1 to CF5), inoculated with 1.5, 3.2, 4.4, 5.6, and 7.2% starter culture, respectively, and incubated at 32°C.

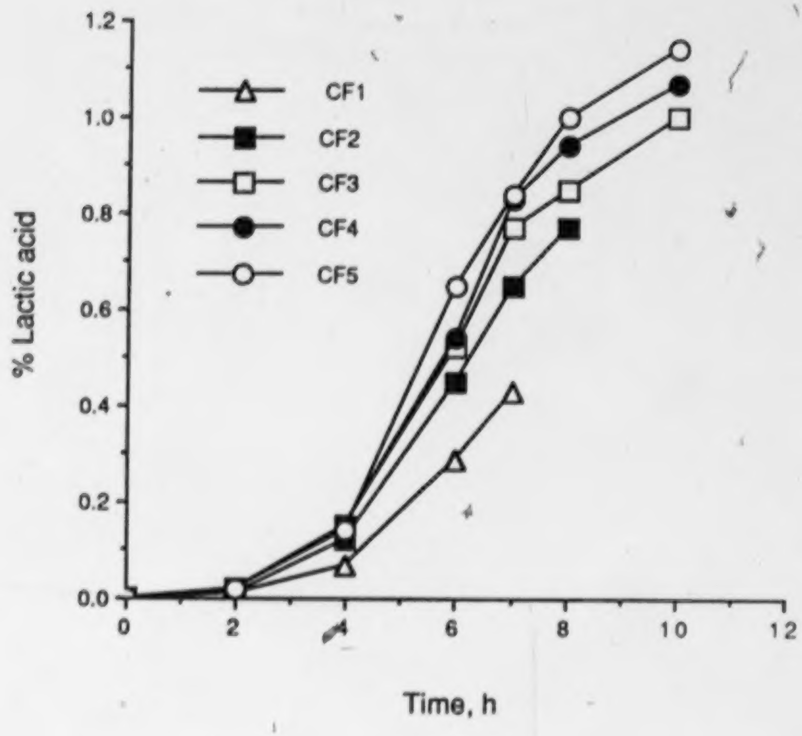

Figure 3.2. Difference in rate of lactic acid production by *L. lactis* subsp. *cremoris* strain 103 in ultrafiltered skim milk of different concentration factors (CF1 to CF5), inoculated with 1.5, 3.2, 4.4, 5.6, and 7.2% starter culture, respectively, and incubated at 32°C until pH 4.6 was attained or for a maximum of 10 h incubation.

CF 5 retentate had a ΔLA of 0.65, about 2.2 times greater than that of CF 1 retentate. The data in Table 3.2 show the doubling times for growth and LA production of *L. lactis* subsp. *cremoris* strain 103. As CF increased, growth rate of strain 103 decreased. However, rate of production of LA increased. Hickey *et al.* (12) reported stimulation of growth and lactic acid production by strains of *L. lactis* subsp. *cremoris* in UF milk retentate of CF 5 by using a starter inoculum of up to 4% (v/v). In this study, larger inocula were used in high CF retentate (the inoculum was proportional to protein content of the retentate) in order to overcome the dB/dpH effect. The results obtained indicate an uncoupling of growth from acid production. Turner and Thomas (37) cited many factors including pH, temperature and high salt concentration that could inhibit growth while lactic acid production continues.

The specific lactic acid production of each strain during logarithmic growth after incubation at 32°C for 6 h is illustrated in Table 3.3. At higher CF retentates, specific lactic acid production increased in all 3 test strains. This indicated that nutritional stress is not the cause but the use of larger inocula might cause stress due to overcrowding of cells which is responsible for the uncoupling of growth from lactic acid production during exponential growth (25).

Although the amount of lactic acid produced increases as the CF is increased, corresponding pH values do not reflect this because of the buffer capacity (dB/dpH). The major constituents responsible for dB/dpH are protein (casein and whey proteins) and minerals, mainly phosphate (15, 34). Ca and Mg ions also influence the dB/dpH because

Table 3.2. Doubling Times for Growth and Lactic Acid Production[a] During Logarithmic Growth of *L. lactis* subsp. *cremoris* 103 Incubated at 32°C Using Starter Inoculum Levels of 1.5, 3.2, 4.4, 5.6 and 7.2% for CF 1 to CF 5, respectively.

CF	Doubling Time for Growth (h)	Doubling Time for Lactic Acid Production (h)
1	1.33	2.74
2	1.52	1.85
3	1.52	1.63
4	2.03	1.54
5	1.90	1.18

[a] Each value represents the mean of 2 replicates.

CF - Concentration factor

Table 3.3. Specific lactic acid production by *L. lactis* subsp. *cremoris* strains 103, 108 and 208 in skim milk and retentates after incubation at 32°C for 6 h using starter inoculum levels of 1.5, 3.2, 4.4, 5.6 and 7.2%, respectively.

CF	Lactic Acid [a] (mM/CFU)		
	Strain 103 ($\times 10^{-7}$)	Strain 108 ($\times 10^{-7}$)	Strain 208 ($\times 10^{-7}$)
1	0.90	3.49	3.83
2	0.91	3.66	4.33
3	0.87	4.13	5.31
4	1.53	4.36	5.40
5	1.87	4.78	5.68

[a] Means of 2 replicates.

CF - Concentration factor

CFU - Colony forming unit

they can form colloidal salts with phosphate which is pH dependent (16). Maximum buffer capacity of the retentates is in the pH range of 5.0 to 5.4 (Fig. 3.3). The maximum dB/dpH value of the retentate increased as a function of increased protein and mineral contents (Table 3.1). Therefore there was less change in pH of higher CF retentate (Fig. 3.4) even though more lactic acid was produced (Fig. 3.2). As a result, pH 4.6 was not achieved in CF 4 and 5 retentates even after incubation for 10 h at 32°C, yet the amount of lactic acid produced in CF 5 is about three times greater than that required for skim milk (CF 1) to reach pH 4.6. The percentage of lactic acid produced by the starter culture strain 103 after 7 h in skim milk (CF 1) was 0.43% (Figure 3.2). It should be noted that the experiment was terminated at this point because a pH of 4.6 was achieved. In CF 5 retentate, the starter culture produced 1.14% lactic acid after 10 h incubation at 32°C (Fig. 3.2). However, the pH of the CF 5 retentate only declined to 4.8 (as shown in Fig. 3.4).

As the pH of the skim milk and retentates decreased to around pH 5.2, coagulation of casein began. This corresponds with the pH of onset of gel formation by acid reported by Heertje *et al*. (11). The isoelectric point of β-casein is at pH 5.2 and at this pH the zeta potential of casein is at a minimum. The pH region where gelation starts also depends on temperature and heat pretreatment of milk (11). The coagulum by acidification was obtained only from CF 1, CF 2 and CF 3 retentates where the isoelectric point (pH 4.6) of casein was reached. Acid coagulation of casein is an important mechanism for yoghurt and cheesemaking.

Proteolytic activity of starter cultures is essential for cell

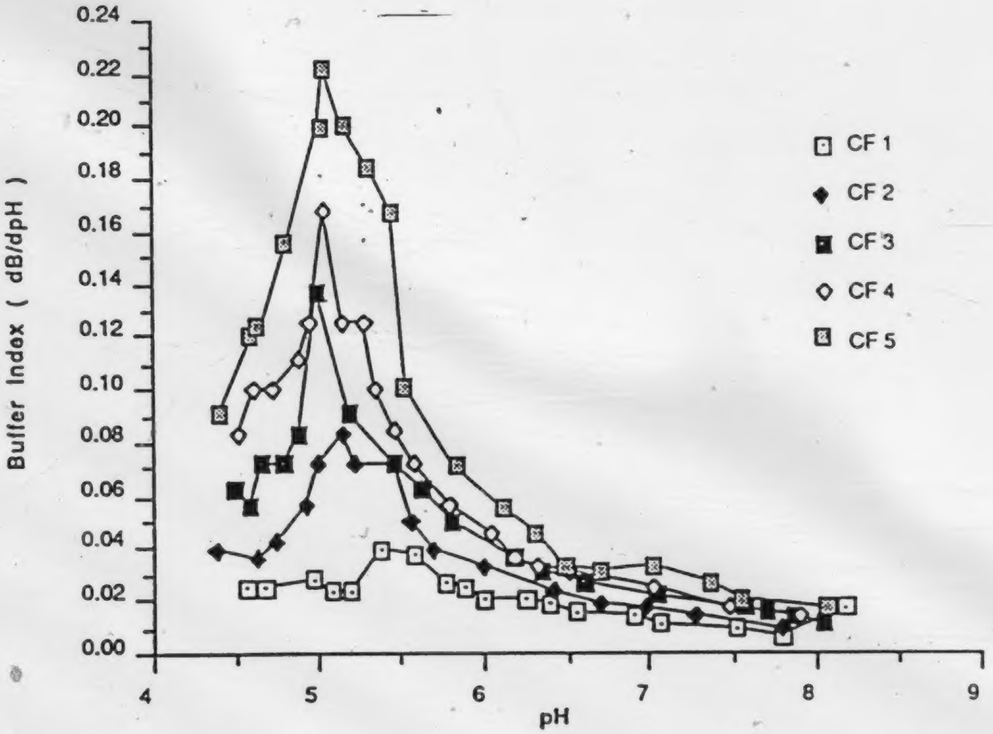

Figure 3.3 Buffer capacity of ultrafiltered skim milk of different concentration factors (CF) determined by titration with 0.1N HCl to pH 4.4 and with 0.1N NaOH to pH 8.

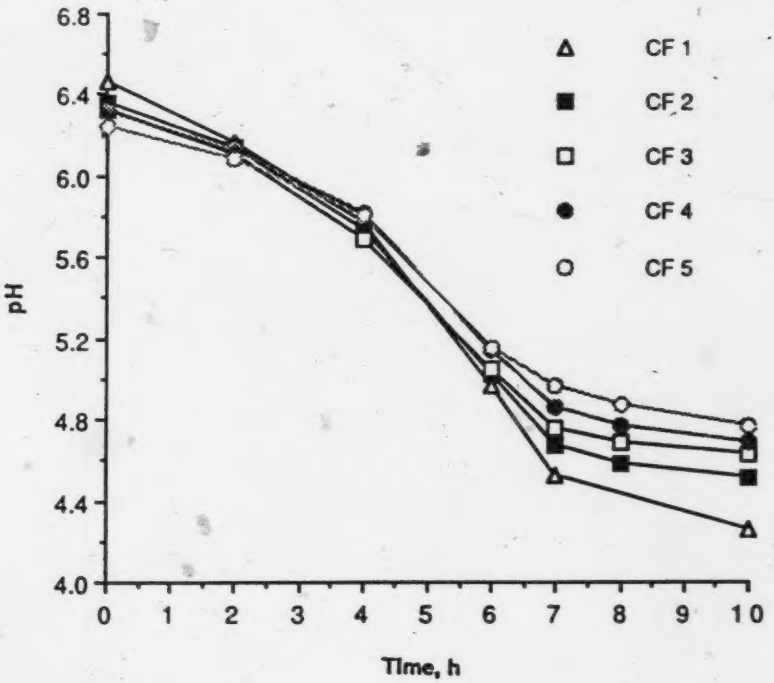

Figure 3.4 Change in pH of ultrafiltered skim milk of concentration factors (CF1 to CF5) by *L. lactis* subsp. *cremoris* strain 103, inoculated with 1.5, 3.2, 4.4, 5.6, and 7.2% starter culture, respectively, and incubated at 32°C.

growth in milk to produce lactic acid at the rate required for manufacture of fermented products. It also plays an important role in rheological and organoleptic properties in cheese ripening (36). Because lactococci are nutritionally fastidious, they require exogeneous supplies of amino acids which are either stimulatory to, or essential for, their growth (21). Amounts of free amino acids and low molecular weight peptides initially present in milk are below the minimum required for active growth. These amino acids are important nitrogen sources for growth up to 8-16% of the maximum cell densities that are found in coagulated milk (35). Peptides of low molecular weight provide another source of amino acids. As cell densities increase, milk proteins become an important source of nitrogen (20, 35). The utilization of milk protein as a source of nitrogen for growth involves bacterial proteinases located in the cell wall and peptidases located in the cell wall, cell membrane and cytoplasm of the starter bacteria (23, 36).

UF of skim milk up to CF 5 resulted in constant levels of non-protein nitrogen (Table 3.1). Thus, there was no change in concentration of free amino acids and low molecular weight peptides available for growth of the starter cultures by UF. The main sources of nitrogen for growth in UF retentates are casein and whey proteins. Thus proteolytic activity is essential to provide a nitrogen source for growth before the concentration of free amino acids and low molecular weight peptides become growth limiting. The proteolytic activity of *L. lactis* subsp. *cremoris* strains 103, 108 and 208 was determined after incubation at 32°C for 6 h. Strain 208 had the highest proteolytic activity whereas strain 103 had the lowest (Table

3.4). Citti et al. (7) showed a direct relationship between proteolysis and acid production in milk by lactic streptococci, this probably accounts for the higher specific acid production of strain 208 compared with strain 103 (Table 3.3) in our study.

The normal temperature for determining activity of starter cultures based upon the increase in titratable acidity or drop in pH, is done at 30-32°C. However, the behavior of starter cultures under cheese manufacturing conditions may differ from that observed under laboratory conditions. Temperature conditions simulating cheese manufacture are recommended for activity tests on starter cultures (10, 13, 29). Therefore, we used 37°C for 5 h to simulate cheddar cheese menufacture for activity tests in UF milk retentates, because during Cheddar cheesemaking the curd temperature is at 37°C for half of the manufacturing time (29). UF milk retentates were inoculated with 0.5, 1, 3, 5 and 10% v/v of freshly prepared bulk starter culture and incubated for 5 h at 37°C, based on the procedure used by Okigbo et al. (27). Initial and final pH were measured and mean change of pH (ΔpH) was calculated. The percent inoculum was plotted on the log scale against the ΔpH using the formula $y = a + b \ln x$ to fit the best straight line to the data, where y = percent inoculum, x = ΔpH, a is the intercept and b is the slope (30). The graph can be used to determine the percent inoculum required for a desired pH change (ΔpH) of different types of cheese. For Cheddar cheese the desired ΔpH is 1.1.

Because of the high proteolytic activity of *L. lactis* subsp. *cremoris* strain 208, an inoculum of only 0.8% was required to give ΔpH 1.1 in CF 1, but in higher CF retentates the percent inoculum

Table 3.4. Proteolytic Activity[a] of *L. lactis* subsp. *cremoris* Strains 103, 108 and 208 after Incubation at 32°C for 6 h

	L. lactis subsp. *cremoris* 103		*L. lactis* subsp. *cremoris* 108		*L. lactis* subsp. *cremoris* 208	
CF	CFU/ml (x 10^8)	µg Tyr/ml	CFU/ml (x 10^8)	µg Tyr/ml	CFU/ml (x 10^8)	µg Tyr/ml
1	3.6	62.88	1.2	33.63	1.0	76.33
2	5.3	79.96	1.4	82.89	0.6	156.56
3	7.8	76.26	2.3	200.17	1.5	237.64
4	4.5	74.67	5.1	245.65	2.4	171.51
5	6.4	60.08	5.5	359.79	2.3	292.95

[a] Each value represents the mean of 2 replicates.

CF - Concentration factor

CFU - Colony forming unit

required for the same ΔpH was higher. At CF 4 an inoculum of 5% was required, while at CF 5 the required ΔpH could not be achieved (Fig. 3.5a), even with an inoculum of 10%. For *L. lactis* subsp. *cremoris* strain 103 which had the lowest proteolytic activity, inoculum required in CF 1 to obtain ΔpH 1.1 was 3.7%. But at CF 2 and higher, the desired ΔpH could not be obtained even with a 10% inoculum (Fig. 3.5b). The proteolytic activity of *L. lactis* subsp. *cremoris* strain 108 was between that of strains 103 and 208 thus the inoculum size required to achieve the desired ΔpH was also intermediate between those strains (Fig. 3.5c). However, a decrease in the rate of acid production in strain 108 at 37°C was observed. Breheny *et al.* (5) found that increasing the incubation temperature from 31.5 to 37°C reduced the growth rate of *S. cremoris* HP but acid production remained about the same, whereas in *S. cremoris* AM2 both acid production and growth rate were reduced with the increase in incubation temperature. A similar effect was seen in our study where acid production by strains 208 and 103 remained relatively unaffected by the change in temperature (i.e. 32°C for normal growth and acid production test, and 37°C for inoculum size determination for cheesemaking), whereas strain 108 required greater inoculum size than would be expected from the starter activity test at 32°C (Table 3.3). Thus the use of conditions simulating those used in cheesemaking is essential to determine the activity and inoculum size of the starter culture for cheesemaking.

It appeared, therefore, that strains with greater proteolytic activity could overcome the high buffer capacity of UF retentate. However, factors other than acid development must be considered in the

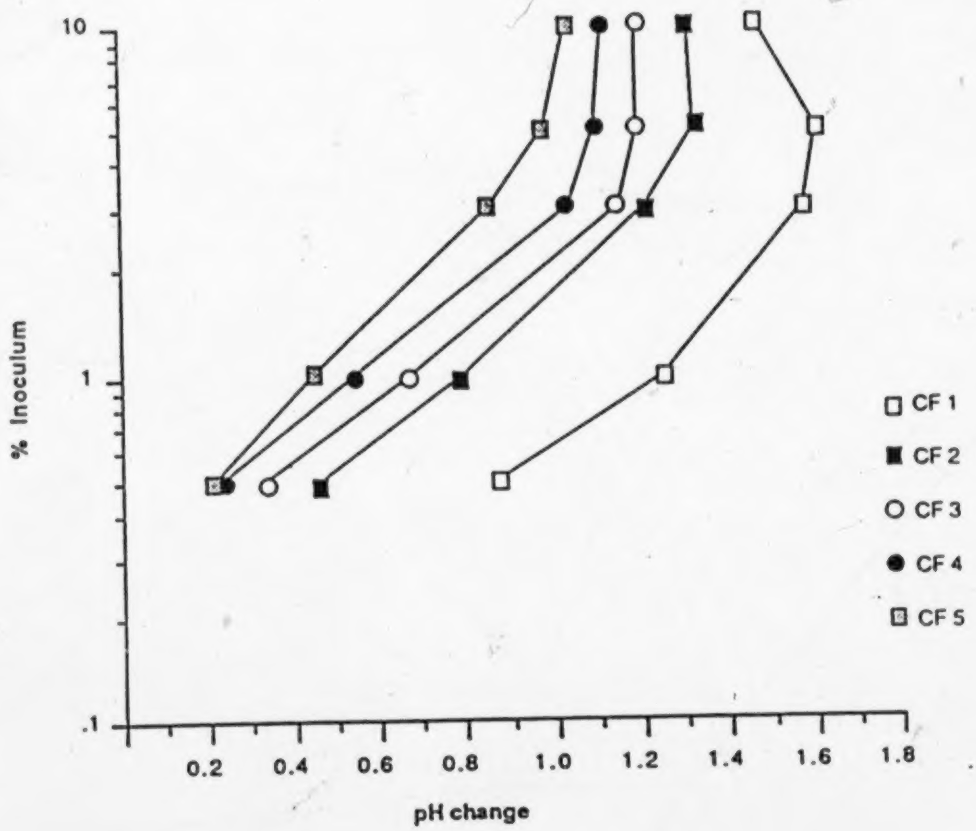

Figure 3.5a Change in pH of ultrafiltered skim milk following incubation for 5 h at 37°C with different concentrations (0.5, 1, 3, 5 and 10%) of starter culture (2.6 X 10^9 CFU/mL) *L. lactis* subsp. *cremoris* strain 208.

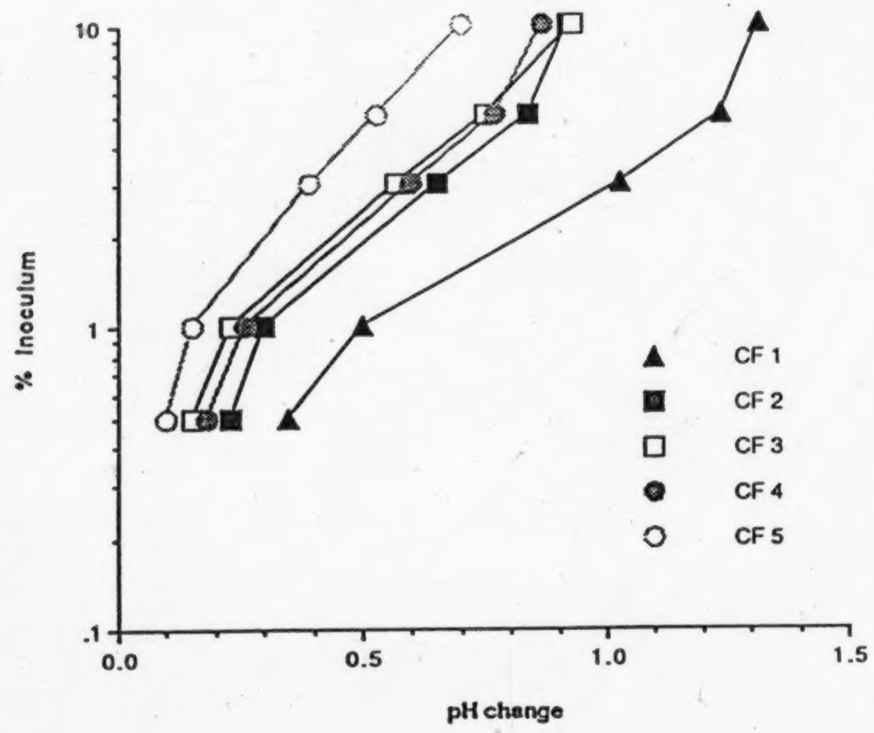

Figure 3.5b Change in pH of ultrafiltered skim milk following incubation for 5 h at 37°C with different concentrations (0.5, 1, 3, 5 and 10%) of starter culture (2.8 X 10^9 CFU/mL) *L. lactis* subsp. cremoris strain 103.

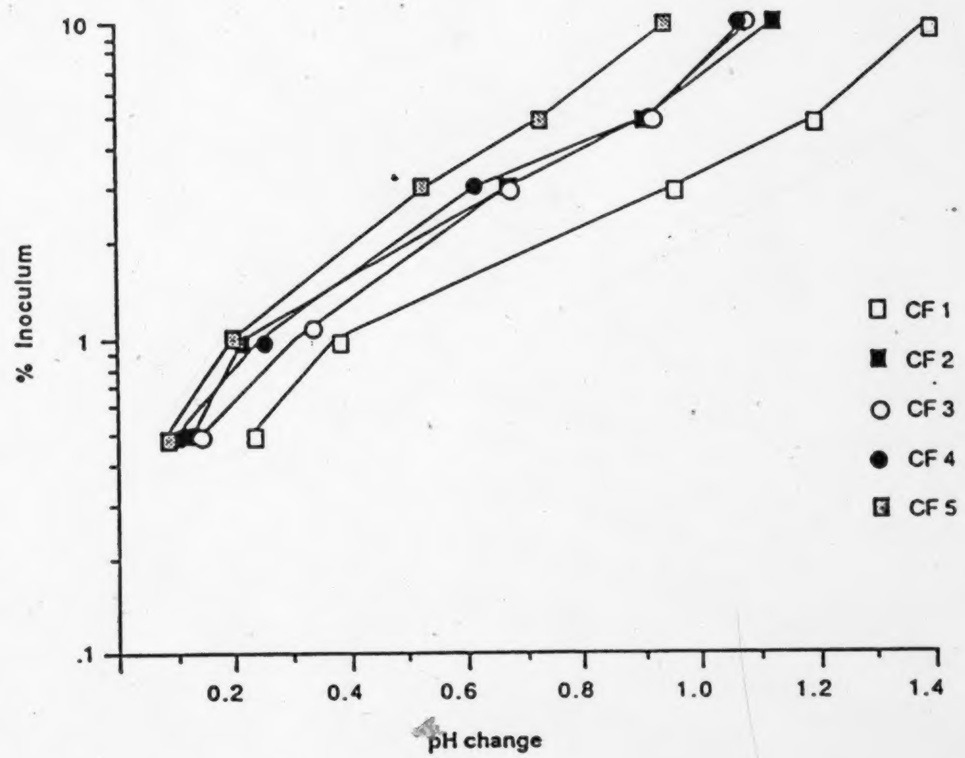

Figure 3.5c Change in pH of ultrafiltered skim milk following incubation for 5 h at 37°C with different concentrations (0.5, 1, 3, 5 and 10%) of starter culture (9.2 X 10^8 CFU/mL). *L. lactis* subsp. *cremoris* strain 108.

selection of a starter strain, for instance the tendency to develop bitter flavor by the formation of a high proportion of hydrophobic peptides from casein in cheese resulting from high levels of starter proteinase (20, 21, 36). Alternative ways to overcome the high buffer capacity include the use of mixed proteinase positive (Prt$^+$) and deficient (Prt$^-$) strains, with the high proportion of the latter (26); or possibly the use of a high concentration of a Prt$^-$ strain (28, 30), which develops less bitterness in cheese made from normal milk, should be tried with the UF milk retentates. Acidifying the milk before UF (34) or coupled with diafiltration (1, 9) reduces the mineral content and successfully overcomes the high buffer capacity effect of UF retentate.

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4. EFFECT OF ULTRAFILTRATION OF SKIM MILK ON CASEIN MICELLE SIZE DISTRIBUTION[1]

4.1 Introduction

The casein in milk is present in roughly spherical particles, called "casein micelles". These micelles contain approximately 94% protein (mainly α_{s1}-, α_{s2}-, β- and κ-caseins) and 6% inorganic matter (principally calcium, phosphate, magnesium and citrate) known as colloidal calcium phosphate (CCP). The CCP plays an important role in association of casein subunits into casein micelles. The properties of these micelles largely determine the stability of milk products during commercial processing such as pasteurization, sterilization, concentration and coagulation (curding) in cheese manufacture (Walstra and Jenness, 1984).

Casein size distribution in milk has been studied extensively. The stability of the milk toward heat and rennet coagulation is a function of casein micelle size (Schmidt, 1980). It is well known that casein micelles of different sizes contain different portions of various types of casein molecules and CCP. Milks containing small micelles are rich in k-casein (Fox, 1982; Sweetsur and White, 1974) and have a low level of CCP (Schmidt, 1980). They are more heat stable than milks containing large micelles which have a low level of k-casein and high content of CCP. Coagulation of milk by chymosin also varies with micelle size. According to Ekstrand et al. (1980) the largest micelles have a longer coagulation time than medium size micelles whereas the coagulation time increases again with the smallest casein micelles. The latter might be due to an increase in the β-casein content which may compete with k-casein for the enzyme, or may dilute the k-casein and disturb the initial bridge formation between the micelles necessary for coagulation (Ekstrand and Larsson-Raznikiewicz, 1978). In addition to the effect of casein micelle size on processing properties, it also affects the rheological properties of the milk products. According to Niki and Arima (1984) the firmness of rennet curd obtained from milk containing small casein micelles is higher than that of milk containing large micelles. However, there is very limited information available on casein micelle size distribution of milk concentrated by ultrafiltration, although UF is a well established unit operation in the dairy industry due to its economic and other advantages. Therefore, the objective of this study was to determine the effect of ultrafiltration on casein micelle size distribution, mean diameter and volume distribution of the casein micelles.

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[1] A version of this chapter has been submitted for publication. S. Srilaorkul, L. Ozimek, B. Ooraikul, D. Hadziyev and F. H. Wolfe. J. Dairy Sci.

4.2 Materials and Methods

4.2.1 Preparation of skim milk retentate

A laboratory ultrafiltration unit (DDS - 20 LAB-Module, Nakskov, Denmark) fitted with a 0.018 m^2 polysulfone membrane (GR 60-PP) with molecular weight cut-off at 25,000 daltons was used for separation and concentration of skim milk retentate. Commercial HTST pasteurized milk was ultrafiltered at 50 ± 1 °C with inlet and outlet pressure of 400 and 320 kPa, respectively. The milk was concentrated to the concentration factors (CF) of 3:1 and 5:1. CF is the ratio of the volume of the original feed to the final volume of the retentate. The skim milk was used as control and referred to as CF 1:1.

4.2.2 Analytical methods

Dry matter and ash content of skim milk and retentate were determined by the AOAC method (AOAC, 1984). Total proteins, casein and non-protein nitrogen (NPN) were determined by macro-Kjeldahl method using a nitrogen to protein conversion factor of 6.38. Whey proteins were calculated from the difference between total protein and the sum of casein and NPN. Fat content was determined by the Mojonnier extraction procedure. The content of lactose was determined by sulfuric acid and phenol colorimetric method as described by Lawrence (1968). Mineral content was determined as follows: P by phosphomolybdate colorimetric method after the sample was ashed (Australian Standard, 1974), Ca and Mg by atomic absorption and K and Na by flame emission spectrophotometry, using Perkin-Elmer Spectrophotometer 4000 (Perkin-Elmer Corporation, Norwalk, CT).

4.2.3. Determination of casein micelle size distribution by transmission electron microscopy (TEM)

Skim milk and ultrafiltered UF skim milk were prepared for TEM by a slight modification of the method of Ali and Robinson (1985). Two mL of ultrafiltered retentate was mixed with 2 mL of 6% glutaraldehyde in 0.2M sodium cacodylate-HCl buffer pH 7.2 and left for 1 h at room temperature. After this initial fixation the mixture was blended with an equal volume of 2.5% molten agar at 60°C, spread on a microscope slide, allowed to solidify and diced into small pieces (1 mm^3). The dices were washed with three changes of 0.2 M sodium cacodylate-HCl buffer pH 7.2 and post fixation carried out by using 1% (w/v) osmium tetroxide in the same buffer. The dices were washed in three changes of deionized water then dehydrated in a graded series of ethanol-water mixtures (50, 70 and 90% ethanol) followed by two washes with absolute ethanol and passed through a series of propylene oxide/Araldite mixture before embedding in Araldite. Thin sections (80 nm thickness) were cut using Reichert-Jung Ultracut E microtome (Reichert Ltd., Vienna, Austria). The sections were stained with lead acetate and uranyl acetate and then examined under an Hitachi 7000 electron microscope (Hitachi Ltd., Tokyo, Japan), at an accelerating voltage of 75 keV. Electron micrographs were taken at a magnification of 30,000X. Three separate blocks of embedded samples were sectioned in order to obtain more representative fields for examination. At least 10 micrographs of casein micelles of each sample were measured separately. Particle size was measured by a Bausch and Lomb measurement magnifier with 7X magnification, then counted and classified into 10 classes, each with a width of 20 nm.

4.2.4 Statistical Methods

Chi square analysis was used to determine the difference in casein micelle size distribution obtained from skim milk and UF skim milk. Analysis of variance and Duncan's multiple range test were applied to determine significant differences among treatment means.

4.3 Results and Discussion

Transmission electron micrographs of casein micelles obtained from skim milk and UF skim milk concentrates are illustrated in Figure 4.1. The casein micelles from skim milk showed roughly spherical shape of various sizes as expected (Figure 4.1A). The appearance of casein micelles from 3- and 5-fold UF skim milk concentrates also exhibited nearly spherical shapes with a wide range of sizes (Figures 4.1B and C). For micelle size distribution in this study, particles less than 20 nm were excluded since they were considered to be submicelles (Mangino and Freeman, 1981; Green et al., 1978; Schmidt and Payens, 1976). Casein micelles with diameters ranging from 20 nm to 358 nm were observed in the skim milk, whereas the 3-and 5-fold concentrates exhibited micelle size of up to 442 and 392 nm, respectively. Caseins with a diameter of up to 680 nm have been reported by McGann et al. (1980).

In this study, casein micelles were classified into 10 classes with 20 nm increments. Micelles larger than 200 nm in diameter were grouped together into one class. The apparent, or observed, size distributions of casein micelles obtained from skim milk and 3- and 5-fold concentrated UF skim milk are presented in Table 4.1. The apparent casein diameters were corrected for the thin sectioning effect in sample preparation for TEM since the plane of sectioning would generally not pass through the center of the micelles. To obtain the true size distribution of casein micelles, a computer program, GW-BASIC (Goldsmith, 1989) was applied. The result of true size distributions of casein micelles of the skim milk and UF skim milk retentates are

Figure 4.1 Transmission electron micrographs of casein micelles from skim milk (A), 3-fold (B) and 5-fold concentrated skim milk (C) at magnification of 30,000X at 75 keV. The bar length indicates 500 nm.

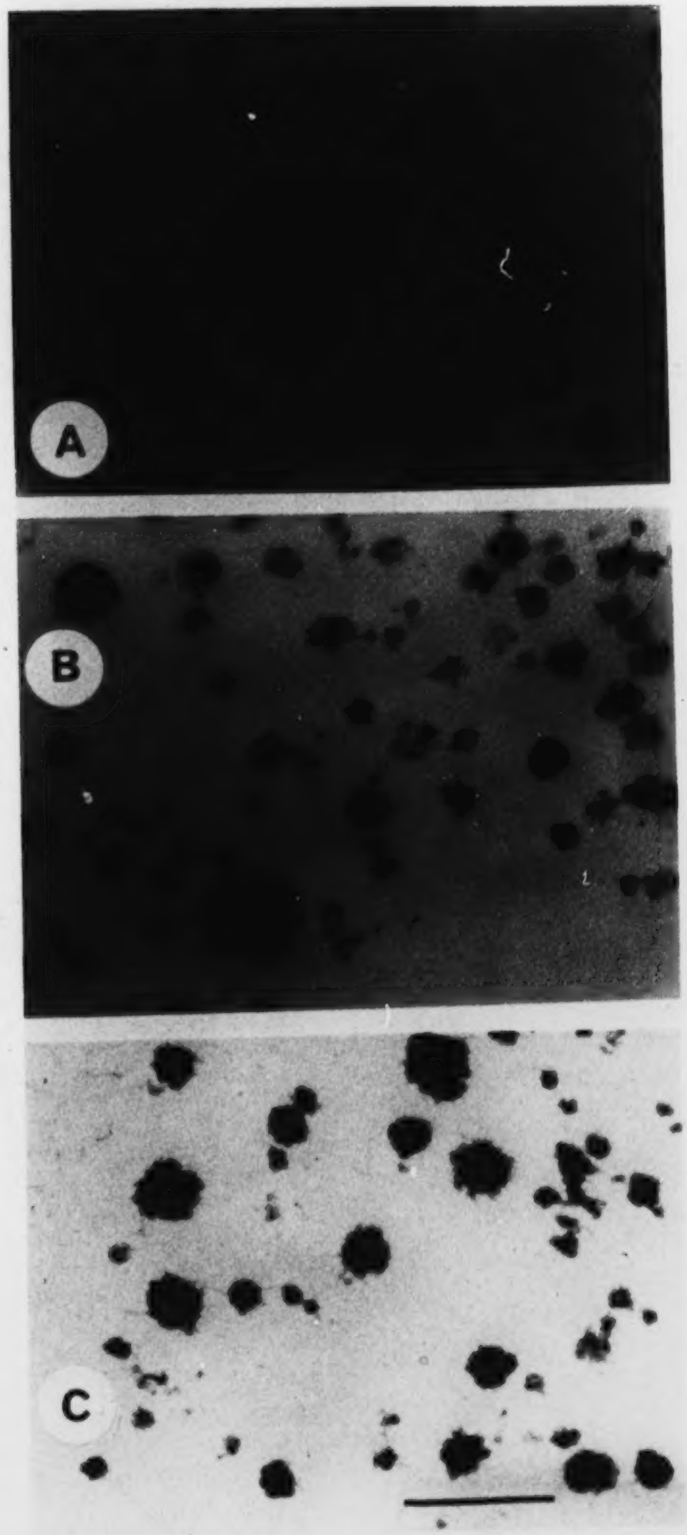
A
B
C

TABLE 4.1. Apparent size distributions, in percentage, of casein micelles in skim milk (CF 1), 3-fold (CF 3) and 5-fold (CF 5) concentrates.

	Casein micelle diameter, nm									
CF	20-40	40-60	60-80	80-100	100-120	120-140	140-160	160-180	180-200	>200
1	2.29	7.35	11.86	21.34	16.21	11.86	11.15	5.69	6.25	6.01
3	2.75	16.75	23.34	25.26	13.68	7.96	5.08	2.24	1.28	1.65
5	7.04	18.18	20.84	20.31	15.03	8.80	4.21	2.45	1.23	1.92

illustrated in Figures 4.2A, B and C.

Chi square analysis of the true casein micelle size distribution of skim milk and the 3- and 5-fold concentrates gave a Chi square value of 43.8 (with 18 degrees of freedom) (Table 4.2). Therefore, there was a highly significant difference ($P < 0.01$) in size distribution of casein micelles obtained from skim milk and ultrafiltered skim milk up to 5-fold concentration. To determine the degree of significant difference of the casein micelle size distribution of the different levels of ultrafiltered concentration from that of skim milk, Chi square values of casein micelle size distribution of 3- and 5-fold concentrates were compared. It was found that Chi square value of 5-fold concentrate (28.03) was higher than that of 3-fold concentrate (24.13). This indicated that there was a higher significant difference in casein micelle size distribution of 5-fold concentrate than 3-fold concentrate when compared with that of skim milk.

The casein micelle size distribution of skim milk exhibited a maximum proportion between a diameter range of 80-100 nm (Figure 4.2A). As the skim milk was ultrafiltered to 3-fold concentrate there was an increase in the proportion of micelles with diameters between 80-100 nm from 24.60% in skim milk to 27.88% in 3-fold concentrate. The proportion of micelles smaller than 80-100 nm also increased significantly ($P < 0.01$), from 9.35% and 6.04% in skim milk to 23.68% and 16.73% in 3-fold concentrate for the ranges of 60-80 and 40-60 nm, respectively (Figures 4.2A and B). Consequently, there was a significant decrease in the number of casein micelles greater than 80-100 nm when compared with that of skim milk. At 5-fold

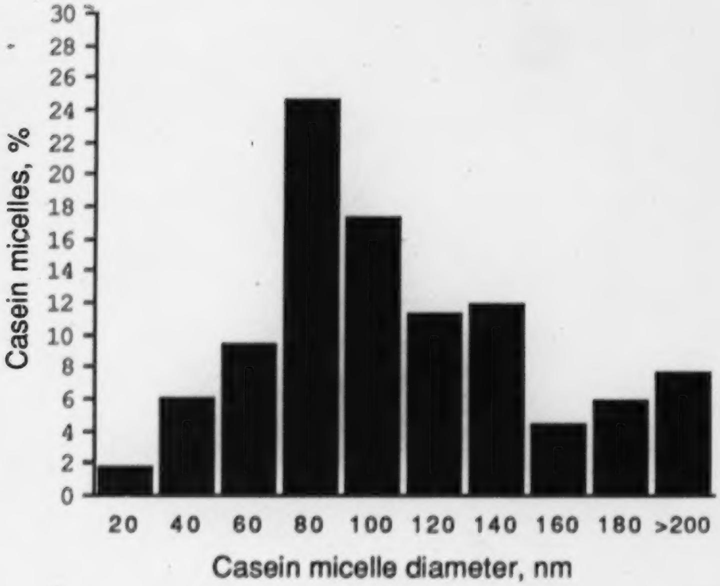

Figure 4.2A True size distribution of casein micelles in skim milk.

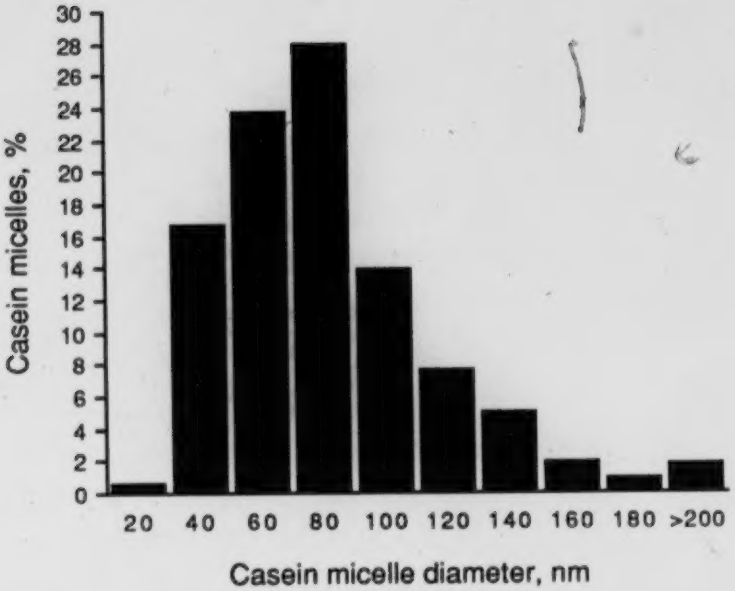

Figure 4.2B True size distribution of casein micelles in 3-fold concentrated skim milk.

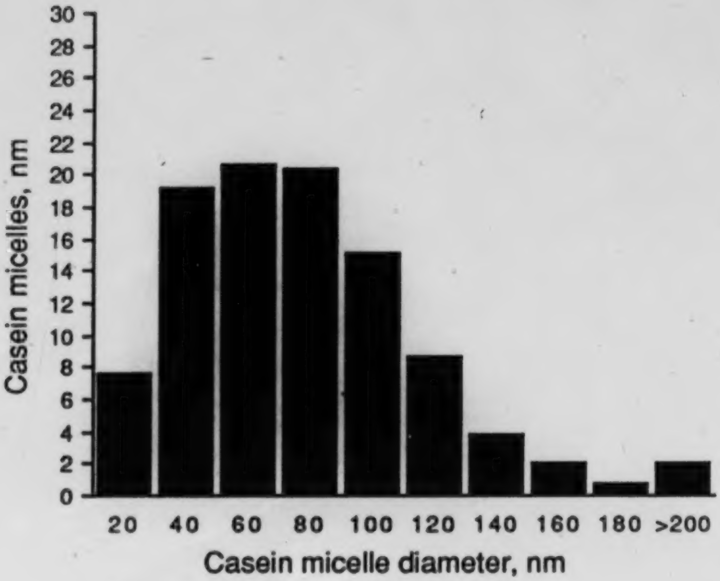

Figure 4.2C True size distribution of casein micelles in 5-fold concentrated skim milk.

TABLE 4.2. Chi square values of casein micelle size distribution obtained from skim milk and ultrafiltered skim milk to 3-and 5-fold concentrates.

Samples Compared	Degree of Freedom	Chi Square Value
CF 1, CF 3 and CF5	18	43.82**
CF 1 and CF 3	9	24.13**
CF 1 and CF 5	9	28.03**

[1] CF 1 - Skim milk; CF 3 - 3-fold ultrafiltered skim milk; CF 5 - 5-fold ultrafiltered skim milk

** Significant at $P < 0.01$

concentrate, the class with maximum frequency was shifted from 80-100 nm in skim milk to 60-80 nm. This resulted in a greater increase in the number of micelles smaller than, and a greater decrease in the number of micelles greater than 80-100 nm compared with CF 1 (Figure 4.2A and C). Therefore, ultrafiltration caused a significant decrease ($P < 0.01$) in average casein micelle size, from 118 nm in skim milk to 92 nm in CF 3 and 87 nm in CF 5 (Table 4.3).

The micelle size distribution may be converted to micellle volume distribution using the following equation (McGann et al., 1980):

$$Vi = \frac{4/3\ \pi\ r_i^3\ n_i}{\Sigma\ 4/3\ \pi\ r_i^3\ n_i} \times 100$$

Where Vi is percentage by volume of casein micelle, ri and ni are the radius and number of casein micelles in class i.

Although the proportion of casein micelles with diameters between 80-100 nm was 24.60% (Figure 4.2A), these micelles occupied only 7.42% by volume of all the micelles (Figure 4.3A) whereas those with diameters of > 200 nm accounted for only 7.61% but they occupied the greatest relative volume of 29.13%. As skim milk was ultrafiltered to 3- and 5-fold concentrates and there was a decrease in the number of micelles with diameter larger than 80-100 nm, as was their volume. The largest relative volume was now occupied by those in the diameter range of 80-100 nm in CF 3 (17.72%) and 110-120 nm in CF 5 (18.19%) (Figures 4.3B and C).

Variations in size of casein micelles and consequently their

Table 4.3. Average mean diameter of casein micelles[1] in skim milk and ultrafiltered skim milk 3- and 5-fold concentrates.

CF	Average micelle diameter, nm
1	118 ± 5.6[a]
3	92 ± 7.7[b]
5	87 ± 5.3[b]

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[1] Obtained from $\bar{X} - \Sigma N_i D_i / \Sigma N_i$ where $\bar{X}$ - average mean micelle diameter, N_i - number of micelles per class, D_i - mean diameter of a given class.

[a,b] Different letters indicate highly significant differences ($P < 0.01$)

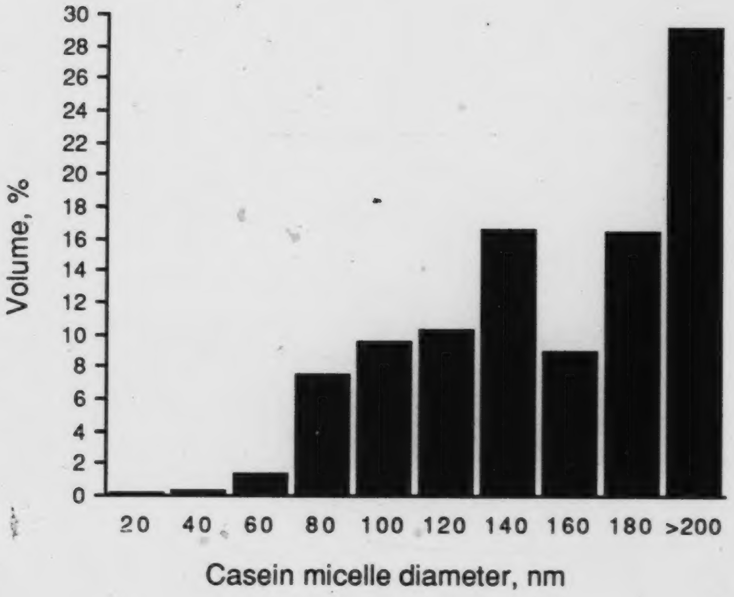

Figure 4.3A Volume distribution of casein micelles in skim milk.

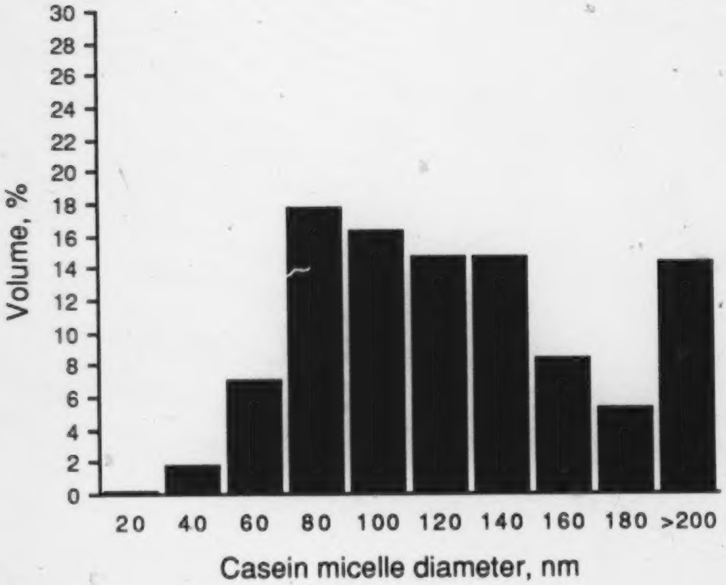

Figure 4.3B Volume distribution of casein micelles in 3-fold concentrated skim milk.

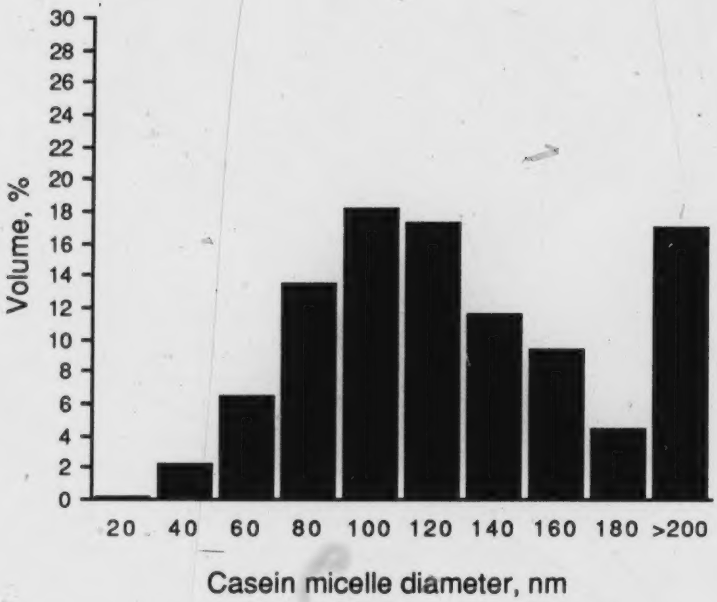

Figure 4.3C Volume distribution of casein micelles in 5-fold concentrated skim milk.

volume distribution and average diameter occur in milk of individual cows (Holt and Muir, 1978; Walstra and Jenness, 1984). Carrol et al., (1968) reported that 75% of the micelles had diameters of 100-170 nm. McGann et al. (1980) showed that the highest number of micelles was at 25-50 nm, with decreasing number as diameters increased. The average diameters of casein micelles in the range of 30 to 120 nm were reported by Mangino and Freeman (1981). McGann et al. (1980) found that the maximum relative volume fraction of casein micelles of skim milk was in the range of 125-150 nm (35% of total volume), while Schmidt et al. (1973) found that it was between 100 and 120 nm (14% of total volume). These variations in normal milk may be caused by the variation in the content of casein and minerals. As the casein micelle size increases the content of k-casein markedly decreases while the content of calcium phosphate increases slightly (Walstra and Jenness, 1984). Therefore, changes in size distribution, average diameter and volume distribution of casein micelles in ultrafiltered skim milk relative to normal skim milk may be mainly due to the change in milk composition as a result of the ultrafiltration process. Macromolecules like casein, whey proteins and fat which are completely retained by the membrane increase in concentration with increasing concentration factor. Consequently, the concentrations of casein, whey proteins and fat in skim milk (CF 1) and 5-fold concentrate (CF 5) were 2.49:12.34:, 0.65:3.06 and 0.11:1.12, respectively (Table 4.4). Smaller molecules and solutes including lactose and minerals can pass through the membrane and hence they are reduced in concentration as concentration factor increases. As a result, the concentrations of Na and K which are mainly in the soluble form are signficantly decreased with increasing concentration factor.

TABLE 4.4. Composition of Skim Milk and Ultrafiltered Skim Milk Concentrates[1].

Constitutents	Concentration Factor		
	1	3	5
Dry matter,%	8.59 ± 0.32	15.69 ± 0.47	22.86 ± 0.01
Casein, %	2.49 ± 0.12	7.37 ± 0.04	12.34 ± 0.40
Whey proteins, %	0.65 ± 0.16	1.82 ± 0.02	3.06 ± 0.08
Fat, %	0.11 ± 0.06	0.49 ± 0.07	1.12 ± 0.12
Non-protein nitrogen, %	0.17 ± 0.01	0.17 ± 0.05	0.21 ± 0.08
Lactose, %	4.97 ± 0.09	4.86 ± 0.05	4.64 ± 0.13
Ash, %	0.77 ± 0.01	1.30 ± 0.03	1.84 ± 0.06
Mineral content (mg/g casein):			
Ca	48.86 ± 0.15^a	42.47 ± 1.57^b	41.71 ± 1.75^b
P	39.11 ± 1.72^a	29.14 ± 0.28^b	26.67 ± 0.82^b
Mg	4.33 ± 0.20^a	2.48 ± 0.08^b	2.09 ± 0.23^b
Na	18.18 ± 1.11^a	7.66 ± 0.28^b	4.78 ± 0.23^c
K	62.71 ± 1.19^a	25.32 ± 0.04^b	17.65 ± 1.90^c

[1] Mean ± SD

[a,b,c] Different letters in the same row indicate significant differences ($P < 0.05$)

For Ca, P and Mg which are partly associated with casein micelles and partly in the soluble form, the concentrations of these minerals decrease significantly from 48.86, 39.11 and 4.33 mg/g casein, in CF 1 (skim milk) to 42.47, 29.14 and 2.48 mg/g casein, respectively in CF 3. The concentrations of Ca, P, and Mg are not further decreased when the CF is increased to 5. This indicated that most of the soluble Ca, P and Mg were removed from the retentates and the remainder was mainly in colloidal form associated with casein. According to Holt and Muir (1978) the average diameter of casein micelles correlates positively with the quantity of colloidal P per unit weight of casein, and there is no relationship between average diameter of the micelles and the concentration of Na, K and Mg. Rose and Colvin (1966) reported that k-casein and soluble Ca were two major factors that influenced casein micelle size. The micelle size distribution can be changed by an addition of k-casein and Ca (Waugh, 1971). However, there was no relationship between average micelle diameter and total Ca, P or Mg content; nor was their a relationship between casein micelle diameter and colloidal Ca content (Rose and Colvin, 1966). Therefore changes in the composition of casein and minerals, particularly Ca and P, will markedly influence the association of casein micelles and aggregation of casein submicelles into casein micelles and, consequently, the size of the casein micelles.

Ultrafiltration of skim milk caused significant changes in size distribution and average diameter of casein micelles. For the micelle size distribution the maximum proportion (24.60% of total micelles) at diameter range of 80-100 nm in skim milk was shifted to the range of

60-80 nm in 5-fold concentrate (20.70% of total micelles). The numbers of casein micelles which were smaller than 80 nm in diameter increased, whereas those which were larger than 100 nm in diameter decreased as the milk was ultrafiltered to higher concentration. Consequently, the average diameter of casein micelles was significantly decreased from 118 nm in skim milk to 92 nm in 3-fold and 87 nm in 5-fold concentrates ($P < 0.01$). Although the proportion of casein micelles at diameter of 80-100 nm was the highest (24.60%), they occupied only 7.42% of the total volume, while the number of casein micelles diameter of > 200 nm was smaller (7.61%) but they contained the largest volume (29.13% of the total volume). As the milk was ultrafiltered the maximum relative volume was shifted from > 200 nm in skim milk to the range of 80-100 nm in 3-fold (17.72%) and 100-120 nm in 5-fold concentrates (18,19%).

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5. MICROSTRUCTURE OF ACID AND RENNET GELS FROM ULTRAFILTERED SKIM MILK[1]

5.1 Introduction

Ultrafiltration has been applied with great success for manufacturing soft and semi-soft cheese varieties. Ultrafiltration of milk to a composition equivalent to that of soft cheese yields a product with acceptable quality in body and texture (Kosikowski, 1986). However, some technical problems still exist in the use of ultrafiltered concentrate directly for the manufacture of hard cheese varieties (solid content of about 60% or higher). Sensory analysis of the product revealed an unacceptable body and texture, i.e. a hard, dry, crumbly and granular body and a coarse texture (Glover, 1985; Shannon, 1987) which corresponded with objective means of instrumental firmness and force to cause fracture (Green et al., 1981). The structure of milk gels determines the retention of fat and moisture which in turn affects cheese yield and composition. Therefore the structure of the milk gel influences the structure, texture, composition and yield of cheese derived from it (Green, 1987). Milk gel structure is affected by many factors, including changes in milk composition and environmental manufacturing conditions. With regard to the organoleptic quality of cheese, texture is more or less of equal importance to flavor (Fox, 1984). Furthermore, the texture is important with regard to certain physicochemical properties, such as stretchability and meltability of Mozzarella type cheeses (Fox, 1984).

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[1] A version of this chapter has been submitted for publication.

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The current study was designed to determine the effect of ultrafiltration of skim milk on the microstructure of acid and rennet gels derived from retentates.

5.2 Materials and Methods

5.2.1 Preparation of Skim Milk Retentate

A laboratory ultrafiltration unit (DDS-20 LAB-Module, Nakskov, Denmark) fitted with a 0.018 m^2 polysulfone membrane (GR 60-PP) with molecular weight cut-off 25,000 daltons was used for separation and concentration of skim milk. Commercial HTST pasteurized milk was ultrafiltered at 50 ± 1°C with inlet and outlet pressure of 400 and 300 kPa, respectively. The milk was concentrated to the concentration factors (CF) of 3:1 and 5:1.

where CF = Volume of original feed/final volume of retentate

The skim milk was used as control and referred to as CF 1:1

5.2.2 Analytical Methods

Dry matter and ash content of skim milk and retentate were determined by the AOAC method (1984). Total proteins, casein and non-protein nitrogen (NPN) were determined by macro-Kjeldahl method using a nitrogen to protein conversion factor of 6.38. Whey proteins were calculated from the difference between total protein and sum of casein and NPN. Fat content was determined by the Mojonnier extraction procedure. The content of lactose was determined by phenol-sulfuric acid method as described by Lawrence (1968). Mineral content was determined as follows: P by phosphomylbdate colorimetric method after the sample was ashed (Australian Standard, 1974); Ca and Mg by atomic absorption; K and Na by flame emission spectrophotometry using a Perkin-Elmer 4000 model spectrophotometer (Perkin-Elmer Corporation, Norwalk, CT).

5.2.3 Preparation of Rennet and Acid Gels

Aliquots of 200 mL skim milk and retentates were adjusted to pH 6.7 and heated to 37°C before 500 μL of 4% solution of chymosin (E.C.3.4.23.4) was added and thoroughly mixed. Coagulation or clotting time (CT) was measured by the hot wire method (Hori, 1985). For microstructure determination, milk gel samples were taken at the time of clotting and then 30 and 60 min after clotting. Acid gel was obtained by acidification of retentate to pH 4.6 with 20% lactic acid at 20°C. Both acid and rennet gels were examined for surface structure by using a scanning electron microscope (SEM).

5.2.4 Determination of Microstructure of Rennet and Acid Gels

Acid and rennet gels were diced (1 mm^3) and fixed for 1 h in 3% glutaraldehyde buffered to pH 7.2 with 0.2 M sodium cacodylate-HCl and postfixed in the same buffer with 1% (w/v) osmium tetroxide for 1 h. After washing in three changes of deionized water, specimens were dehydrated in a graded series of ethanol-water mixtures (50, 70 and 90% ethanol) and twice with absolute ethanol then passed through a series of propylene oxide/Araldite resin mixtures before embedding in

Araldite. Sections (1-2 μm thick) were cut by microtome (Reichert-Jung Ultracut E, Vienna, Austria). The sections were placed on a drop of deionized water on a circular 12 mm diameter glass cover slip, stretched and dried by gentle heating on a hot plate. These sections were then subjected to resin removal by etching in ethanol solution containing NaOH by the method of Brooker and Wells (1984), then the cover slips with specimens were transferred rapidly to ethanol and washed several times before being air dried. The cover slips were mounted on Al-stubs by using silver conducting paste and then sputtered with gold in a sputter coater (Edward S 150B, Sussex, U.K.). The specimens were examined by Cambridge M250 (Cambridge, U.K.) and Phillips 505 (Eindhoven, The Netherlands) scanning electron microscopes at an accelerating voltage up to 30 keV.

5.3 Results and Discussion

The composition of skim milk (CF 1) and ultrafiltered skim milk retentates (CF 3 and CF 5) used in this study are shown in Table 5.1. The contents of dry matter and macromolecules like casein, whey proteins and fat increased proportionally with increased concentration factor (CF). As the CF increased, non-protein nitrogen (NPN) remained fairly constant while there was a slight decrease in the content of lactose. Mineral content (Ca, P, Mg, K and Na) increased linearly with an increase of CF and there was also a rise in ash content.

Scanning electron micrographs of milk gels obtained by coagulation of skim milk (CF 1) and retentates (CF 3 and CF 5) are presented in Figures 5.1a,b-5.4a,b, respectively. The lower magnifications (Figures

Table 5.1 Composition of skim milk and ultrafiltered skim milk concentrates[1]

Constituents	Concentration Factor		
	1	3	5
Dry matter, %	8.59 ± 0.32	15.69 ± 0.47	22.85 ± 0.01
Casein, %	2.49 ± 0.12	7.37 ± 0.04	12.34 ± 0.40
Whey proteins, %	0.65 ± 0.16	1.82 ± 0.02	3.06 ± 0.08
Fat, %	0.11 ± 0.06	0.49 ± 0.07	1.12 ± 0.12
Non-protein nitrogen, %	0.17 ± 0.01	0.17 ± 0.05	0.21 ± 0.08
Lactose, %	4.97 ± 0.09	4.86 ± 0.05	4.64 ± 0.13
Ash, %	0.77 ± 0.01	1.30 ± 0.03	1.84 ± 0.06
Mineral Content (mg/100g):			
Ca	121.66 ± 4.87	312.98 ± 9.79	514.64 ± 4.72
P	97.38 ± 0.67	214.76 ± 3.20	329.08 ± 0.59
Mg	10.77 ± 0.09	18.26 ± 0.49	25.82 ± 0.47
Na	45.26 ± 1.07	56.50 ± 1.14	59.01 ± 2.27
K	156.14 ± 1.12	186.55 ± 1.34	217.81 ± 5.90

[1] Mean ± SD

5.1a-5.4a) provide the structure of the whole network of the milk gels, whereas higher magnification (Figures 5.1b-5.4b) provide more detailed information of the structural changes that occur at the casein micellar level.

Rennet (Chymosin) Gels

The micrograph of rennet gels prepared from CF 1 revealed a considerable change in the network structure with time after addition of rennet (Figures 5.1a,b). At the start of coagulation, designated rennet coagulation time (RCT), some of the casein micelles were bridged or linked together forming chains oriented in all directions. In addition, some micelles were grouped into clusters. Some of these clusters and chains were then interconnected or cross-linked initiating the formation of a three-dimensional network structure. As revealed at high magnification (20,000X), the spherical structure of casein was maintained at RCT. At 30 and 60 min after RCT more chains and cross-linkages were formed. As a consequence, smaller pores, compartments or void spaces were created. The average diameter of the pores for skim milk (CF 1) were 5.7 μm at RCT, 3.8 μm at 30 min and 3.3 μm at 60 min after addition of rennet. These phenomena were accompanied with simultaneous fusion of the casein micelles. The extent of fusion and increase in micelle size followed the time elapsed after the addition of rennet (Figure 5.1). Thus, the micellar size was larger in gels obtained at 60 min (average diameter was 185 nm) than in gels kept for 30 min (average diameter was 148 nm) after RCT; the micelles at the start of coagulation were the smallest (average diameter was 110 nm; see Figure 5.1b at a magnification of 20,000X).

Figure 5.1a Scanning electron micrographs of rennet gels obtained from skim milk at rennet clotting/coagulation time (RCT) (A), 30 min (B) and 60 min after RCT (C) at a magnification of 1,550X at 30 keV acceleration. (D) is the same as (C) but at a magnification of 5,000X at 30 keV acceleration. The bar lengths are 10 μm.

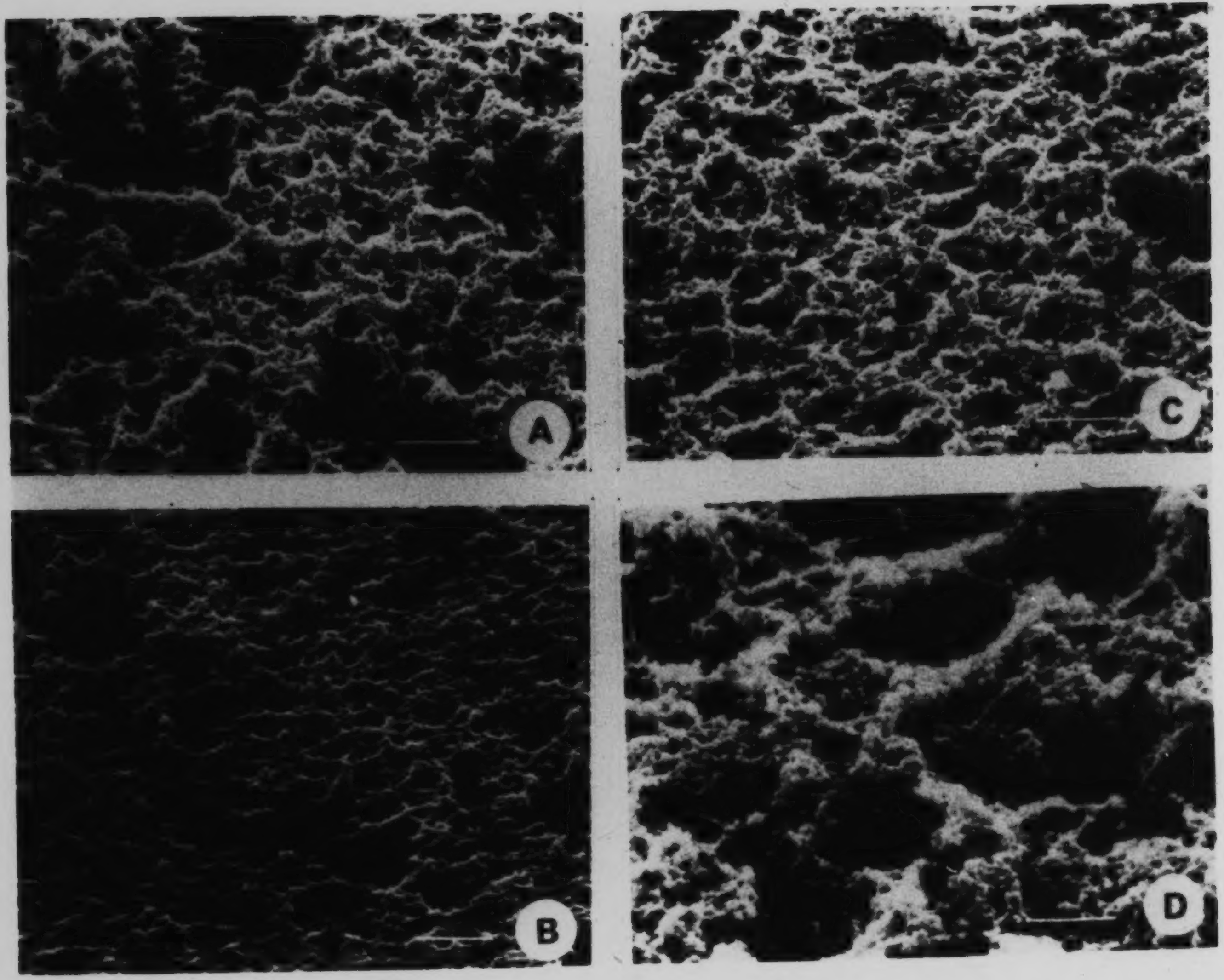
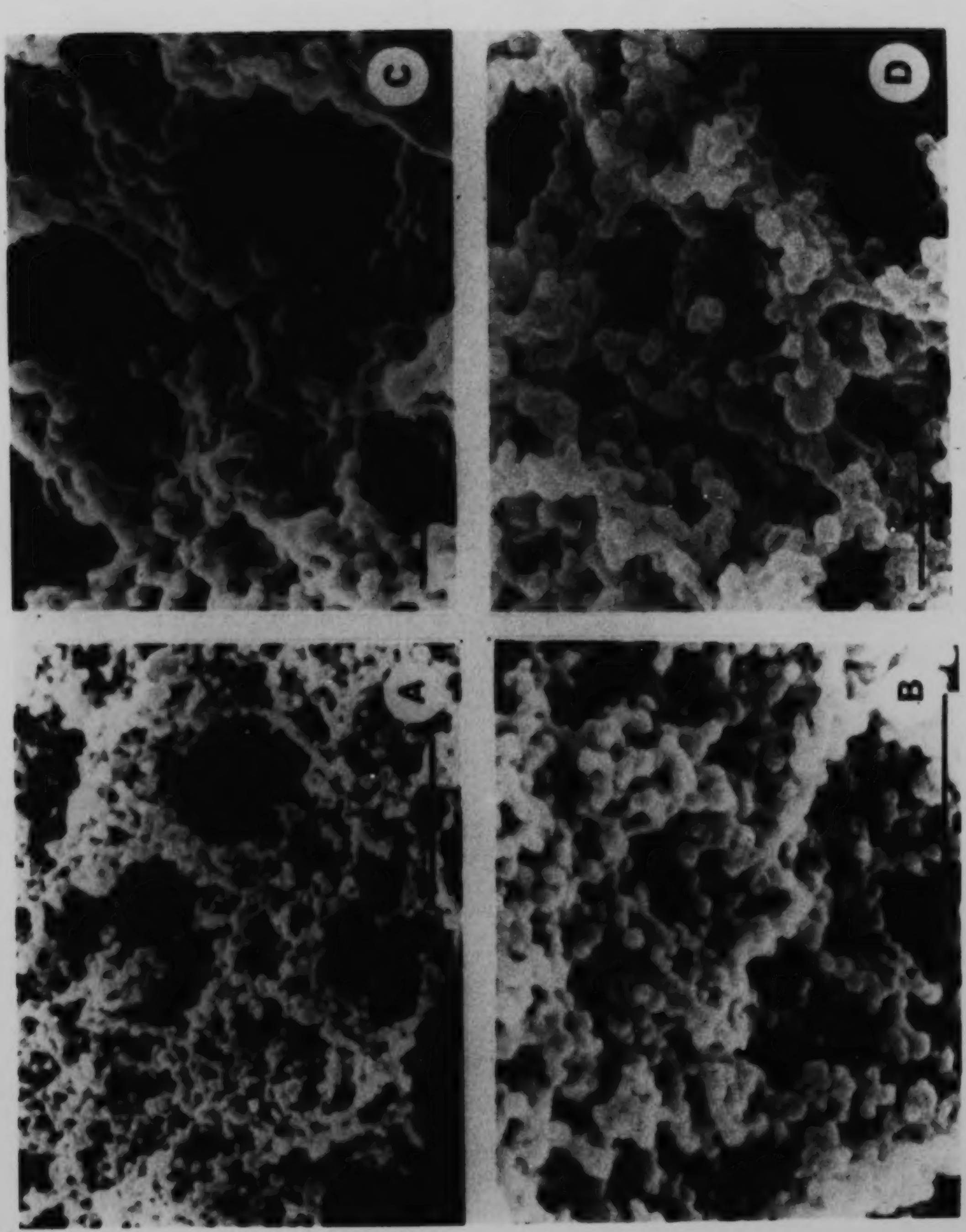

A
C
B
D

Figure 5.1b Scanning electron micrographs of rennet gels obtained from skim milk at rennet clotting time (RCT) (A,B), 30 min (C) and 60 min after RCT (D) at a magnification of 10,000X (A) and at a magnification of 20,000X at 20 keV acceleration (B, C, D). The bar lengths are 2 (A) and 1 μm (B,C,D). .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The mechanism of gel formation and its network structure are not fully understood (Kalab and Harwalkar, 1973). The structure of the gels obtained in our study was similar to that described by Brooker (1979). The author used transmission electron microscopy to observe the changes in microstructure of milk gels after rennet addition.

Based upon all these observations it might be assumed that the first step in development of gel structure involves aggregation of individual casein micelles followed by chain formation. The chains of variable lengths and directions are formed by direct contact of micelles or by bridges generated between the micelles. The gel is formed when these chains grow in size and cross-link to form a loose three-dimensional network that holds the entrapped liquid or milk serum. When such a gel is left undisturbed there is a continuous increase in number and strength of linkages between casein micelles. Consequently, casein strands become shorter and thicker and finally they fuse together into a large lump of an amorphous shape.

Similar changes in casein micelles were observed to occur during cheddar cheesemaking (Green and Grandison, 1987). The casein micelles aggregate with a loss of micelle identity that results in a steady increase in the micelle fusion into an amorphous mass. The mass size increases and finally provides the continuous network structure of the cheese.

Several forces are involved in casein micelle coagulation by rennet. Hydrophobic interactions are involved, as evidenced from temperature-dependent coagulation of rennet treated casein. Electrostatic interactions involve calcium crosslinking of caseins. These interactions directly affect the negatively charged casein residues and indirectly enhance protein hydrophobic interactions (Bringe and Kinsella, 1987; Walstra et al., 1985).

Rennet gels obtained from ultrafiltered skim milk retentates (CF 3 and CF 5) also change with time after addition of rennet (Figures 5.2a,b and 5.3a,b). In this case, due to the high concentration of casein micelles and low moisture content, the gel network obtained from CF 3 (casein, 7.37 ± 0.04; and of dry matter, 15.69 ± 0.47%) was tightly packed with distinct characteristics of having small cracks with an average crack width of 0.27 μm and 1.63 μm length. These cracks appear throughout the whole network (Figure 5.2) but are not present in skim milk gel (CF 1; casein, 2.49 ± 0.12%; dry matter content, 8.59 ± 0.32%). These cracks or narrow void spaces were located at the sites where the casein micellular chains were formed during RCT. As expected, these cracks were much more pronounced in CF 5 at RCT (Figure 5.3). Here the casein concentration was the highest i.e. 12.34%. The cracks were curved and had an average length of 5.2 μm and a width of 0.5 μm.

The differences in the rennet gel structures observed between CF 3 and CF 5 samples from that of skim milk CF 1 at the rennet coagulation time may be due to a decrease in the mean distance of casein micelles as the casein concentration increased. According to Green et al. (1981), the mean free distance of casein micelles is reduced from three micelle diameters in normal milk to less than one micelle diameter in a 4-fold concentrated milk. An increase of casein concentration results in restricted movement of casein micelles, hence a restricted choice of positions for micelles, and restricted types of gel structure.

In addition, an increase in milk protein concentration may

Figure 5.2a Scanning electron micrographs of rennet gels obtained from 3-fold concentrated skim milk at rennet clotting time (RCT) (A), 30 min (B) and 60 min after RCT (C) at a magnification of 1,550X at 30 keV acceleration. (D) is the same as (C) but at a magnification of 5,000X at 30 keV. The bar lengths are 10 μm.

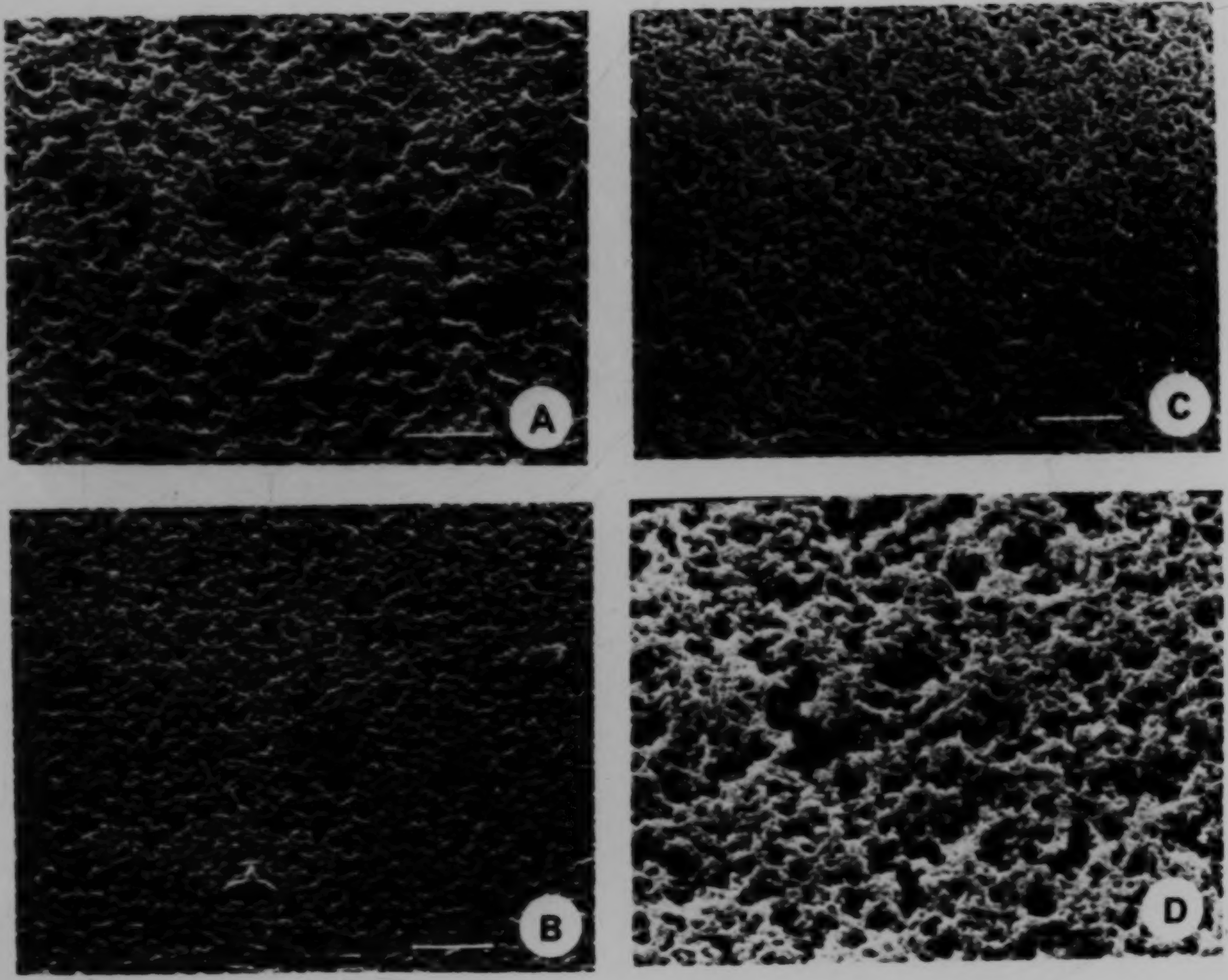
A
C
B
D

Figure 5.2b Scanning electron micrographs of rennet gels obtained from 3-fold concentrated skim milk at rennet clotting time (RCT) (A), 30 min (B) and 60 min after RCT (C,D) at magnification of 10,000X at 15 keV acceleration (C) and at magnification of 20,000X at 20 keV acceleration (A,B,D). The bar lengths are 1 (A,B,D) and 2 μm (C).

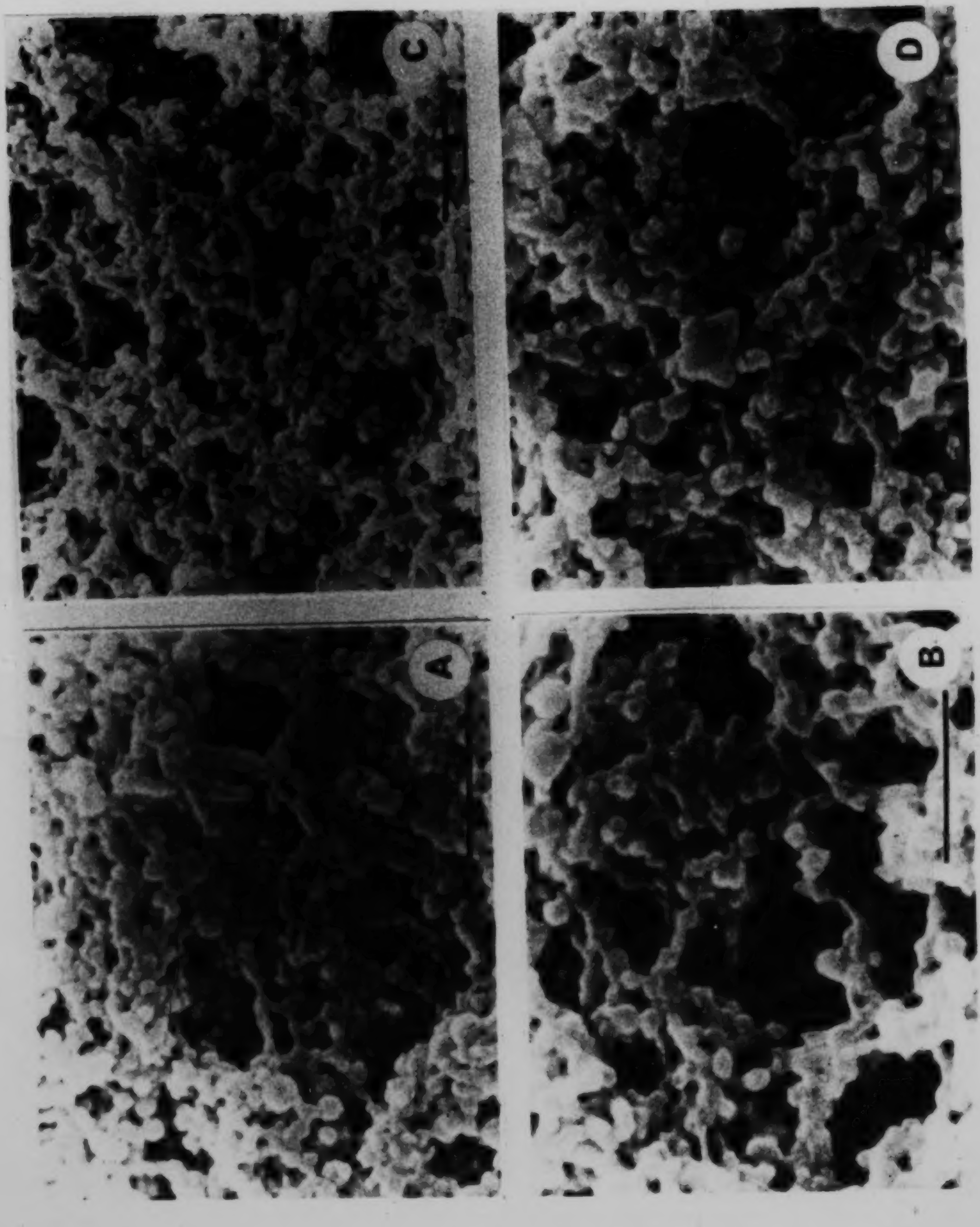
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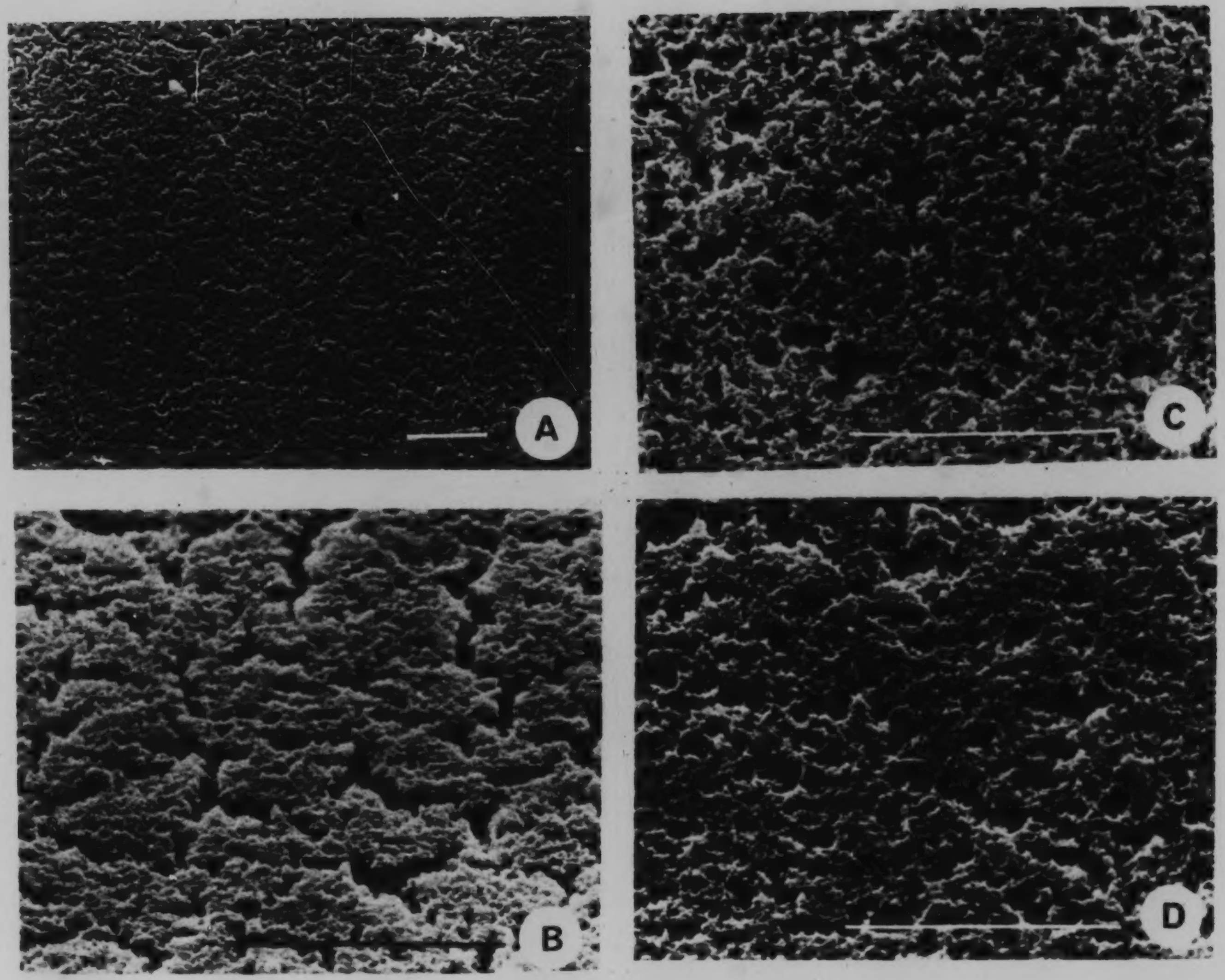
Figure 5.3a Scanning electron micrographs of rennet gels obtained from 5-fold concentrated skim milk at rennet clotting time (RCT) (A,B), 30 min (C) and 60 min after RCT (D) at a magnification of 5,000X at 30 keV accelelation. (A) is the same as (B) but at a magnification of 1,550X at 30 keV acceleration. The bar lengths are 10 μm.


A
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B
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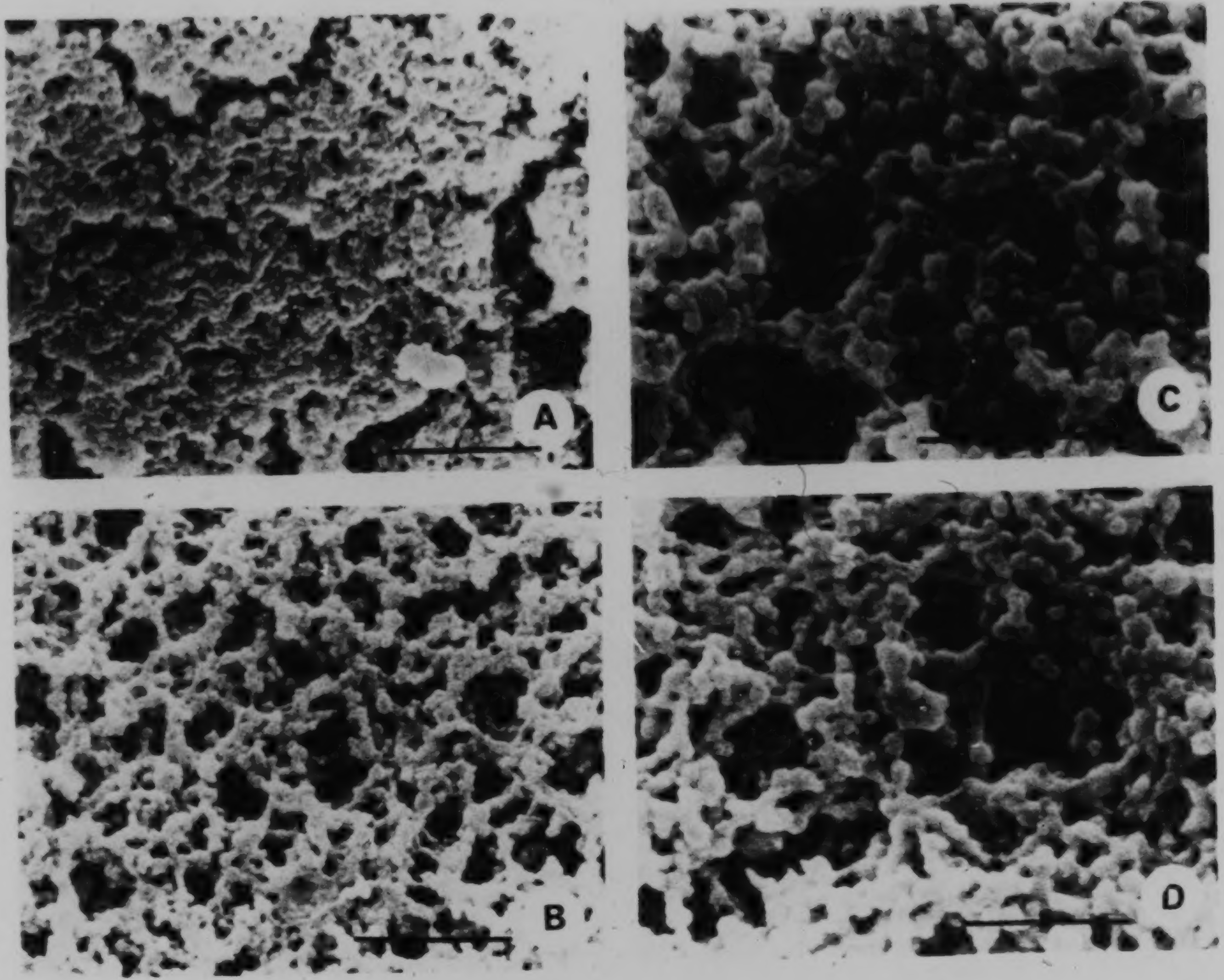
Figure 5.3b Scanning electron micrographs of rennet gels obtained from 5-fold concentrated skim milk at rennet clotting time (RCT) (A), 30 min (B,C) and 60 min after RCT (D) at a magnification of 10,000X at 20 keV acceleration (A,B) and at a magnification of 20,000X at 20 keV acceleration (C,D). The bar lengths are 2 (A,B) and 1 μm (C,D).


A
C
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influence the gel structure by altering the mechanism of casein micelle aggregation. According to Dalgleish (1981) about 90% of casein in normal milk is incorporated into the curd at RCT. As milk concentration is increased the amount of casein incorporation is decreased at the RCT-point. Thus, only about 50% of casein is incorporated into the curd of a 4-fold milk concentrate at RCT. Fox (1984) reported that micelles that are free at RCT will later be incorporated into the gel differently by reacting with either the existing gel matrix or with existing free casein micelles. This suggests a possible involvement of a two-step process in gel assembly (Fox, 1984).

The quantity of free micelles at RCT may markedly affect the properties of the final gel. This may explain the atypical structure of the rennet gel obtained from high skim milk retentates of CF 3 and CF 5 as observed in our SE-micrographs. However, when more time was allowed, as in samples 30 min after RCT, more casein micelles were incorporated into the gel network. As a result of time elapsed, the interspatial cracks disappeared in both the CF 3 and CF 5 network (Figures 5.2 and 5.3). The average diameter of casein micelles for CF 3 and CF 5 at 30 min after RCT were 137 and 130 nm, while the average pore sizes were 1.14 and 0.78 μm, respectively. At 60 min after RCT a more uniform network was obtained, with smaller pore sizes averaging 0.94 and 0.63 μm and with an increased casein micelle size averaging 148 and 140 nm for CF 3 and CF 5, respectively. Incomplete incorporation of casein micelles into the gel matrix may be an important event and may partly explain the unsatisfactory texture of many hard cheese varieties (Fox, 1984).

Acid Gels

Since major consituents of milk casein (α_{s1}-, α_{s2}-, β- and κ-caseins) have different isoelectric points, acidification of milk to pH 4.6 at 20°C is applied as a compromise to yield the optimum aggregation of casein (Graham, 1976; Roefs et al., 1985). However, some reports indicate that the isoelectric point of casein micelles is about pH 4.6 (Bringe and Kinsella, 1987; Walstra and Jenness, 1984). Micrographs of acid gels are shown in Figures 5.4a,b.

When acid gels are compared with rennet treated gels (Figures 5.1-5.3) it appears that acid gels made from skim milk and ultrafiltered skim milk retentates have similar structures to the rennet gels of corresponding concentration (CF) at 60 min elapsed after RCT. However, this type of structure did not occur before this time was reached, a finding similar to that reported by Knoop and Peters (1975) when the microstructure of rennet and acid gels from normal milk were analyzed by transmission electron microscopy. These findings strongly suggest that the network of acid gels from skim milk and ultrafiltered retentates very rapidly reach an end point structure as a result of rapid acid neutralization of the negative micellar charge. Thus, a net zero charge on casein micelles is established and no further change occurs in the network structure as a function of time (Knoop and Peters, 1975). Roefs et al. (1985) also reported the same microstructure for acid and rennet gels obtained from normal milk. These findings are rather surprising since acid and rennet coagulations involve totally different mechanisms.

Casein coagulation by acid treatment occurs as the result of a rapid reduction of steric repulsion by hydrogen ions (Bringe and

Figure 5.4a Scanning electron micrographs of acid gels obtained from skim milk (A), 3-fold (B) and 5-fold concentrated skim milk (C) at a magnification of 3,100X at 30 keV. (D) is the same as (B) but at a magnification of 5,000X at 30 keV acceleration. The bar lengths are 10 μm.

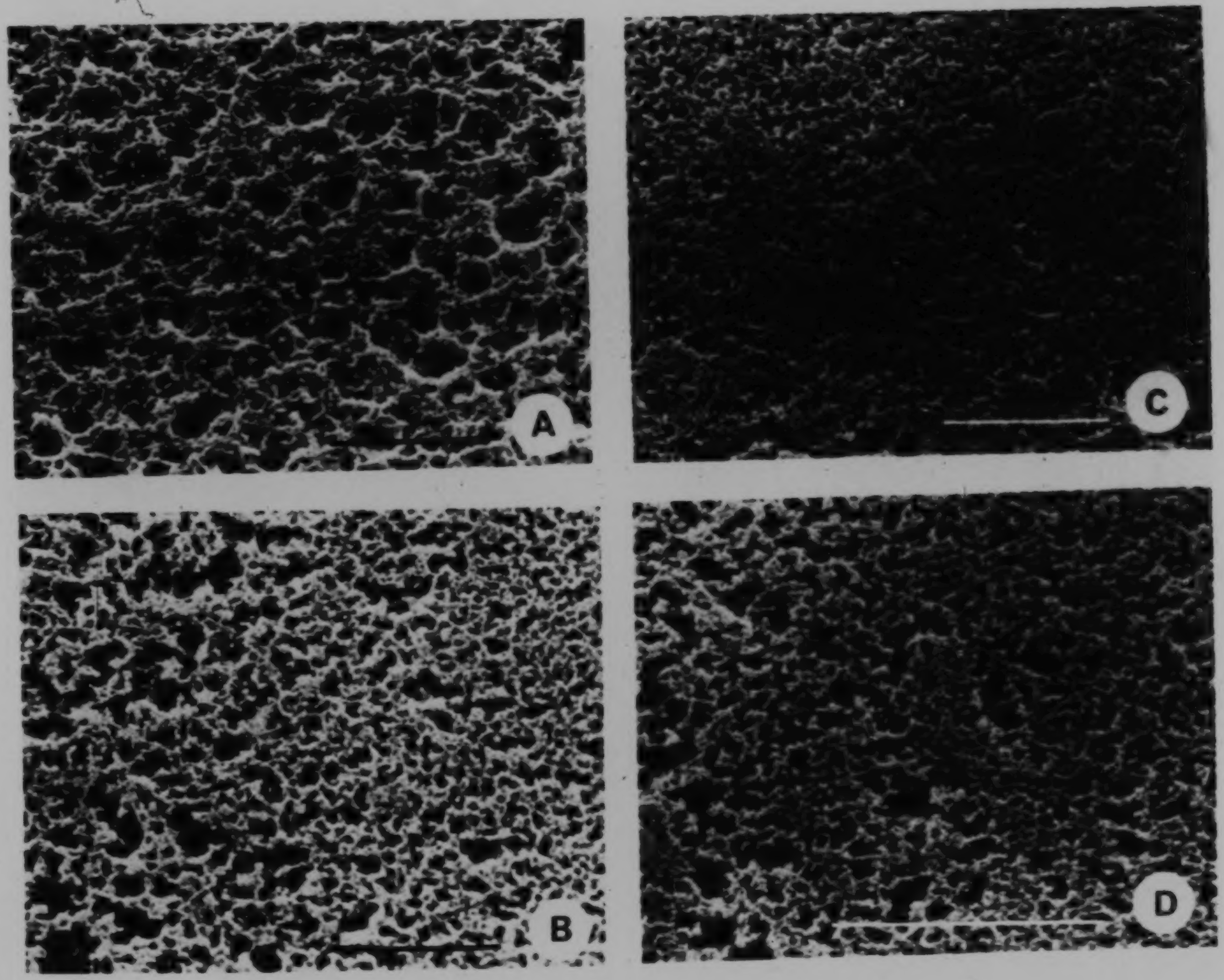
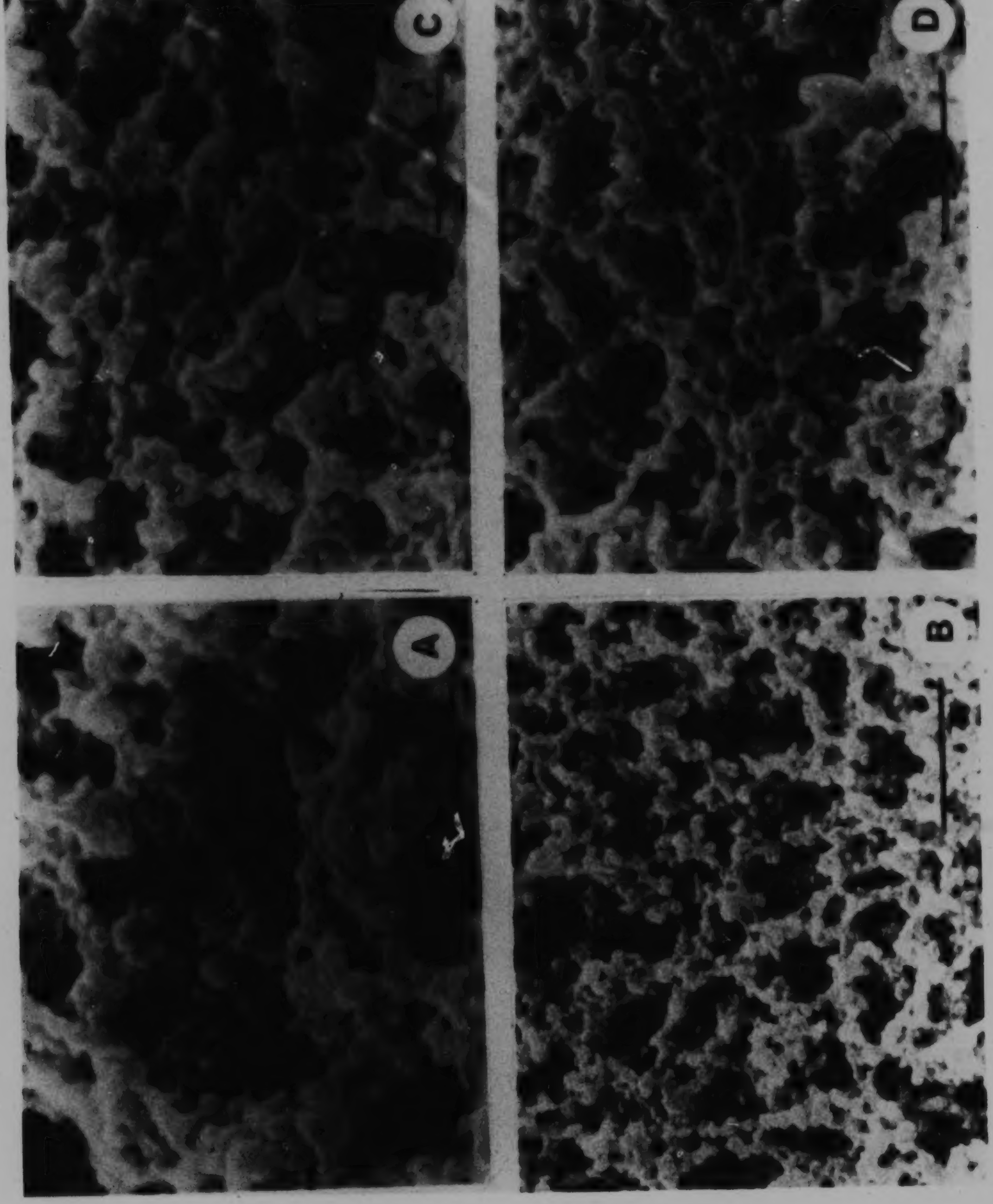

A
C
B
D

Figure 5.4b Scanning electron micrographs of acid gels obtained from skim milk (A), 3-fold (B,C) and 5-fold concentrated skim milk (D) at magnification of 10,000X at 20 keV acceleration (B) and a magnification of 20,000X at 20 keV acceleration (A,C,D). The bar lengths are 1 μm except on micrograph (B) where the length is 2 μm.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Kinsella, 1987) and a rapid reduction of the negatively charged casein to its isoelectric pH, i.e. when the net charge of casein is zero. However, according to Heertje et al. (1985), the aggregation of casein micelles into curd or gel during milk acidification is more than just aggregation of the original casein micelles. It involves dissolution of colloidal calcium phosphate (CCP) which acts as a cementing agent for the integrity of casein micelles. Therefore, acidification causes a process of micellar disintegration particularly as a result of β-casein leaving behind a micellular framework of α-$_s$-casein which remains intact upon lowering the pH especially to a range of pH 5.5-5.2. At the isoelectric point of β-casein (pH 5.2), it starts to aggregate. CCP is completely removed from the micelles at pH 5.0. Upon further lowering the pH to 4.6, β-casein which has now acquired a positive charge, may be reabsorbed onto the micellar framework consisting of α-casein, which is still negatively charged at this pH. Consequently, a coagulum formed from newly formed casein particles differs in structure and composition from a coagulum derived from original micelles. Hence the acid gel obtained is granular in structure, is extremely fragile and is very inelastic due to a lack of calcium (De Hasst, 1982; Johnson, 1988).

As already mentioned, milk coagulation by rennet involves two phases (Fox, 1986; Dalgleish, 1987). The primary or enzymatic phase involves hydrolysis of k-casein at Phe_{105}-Met_{106} bond by rennet with the release of a highly charged hydrophilic macropeptide. As a result of this enzymatic attack there is a decrease in the zeta potential of casein micelles and therefore reduction in electrostatic repulsion. In addition removal of protruding peptides from the micellar surface reduces steric repulsive forces.

The second phase of rennet induced milk coagulation involves a nonenzymatic coagulation step when about 85% of the total κ-casein has been hydrolyzed. Here the casein micelles begin to aggregate to form a gel at temperatures higher than 18°C. Aggregation is achieved in the presence of Ca-ions which act either as cross-linking cations for casein micelles or simply as a charge neutralizer. Consequently, in a rennet gel incorporation of casein micelles into a chain is slower than in acid-treated micelles due to a slower drop in electrostatic charge of the micelles (Knoop and Peters, 1975; Kalab, 1979).

Nevertheless, the acid gels obtained in this study revealed a denser network and thus smaller pores than rennet gels of corresponding concentration (CF) at 60 min time after RCT. Acid gel pores averaged 1.28, 0.33 and 0.26 μm diameter, whereas the rennet gels were 3.3, 0.94 and 0.63 μm for CF 1, CF 3 and CF 5, respectively. The size of casein micelles in acid gels was also smaller than that in rennet gels of the corresponding concentration at the same time, i.e. 60 min after RCT. Thus the average micelle sizes of acid gels were 150, 130 and 110 nm, whereas those of rennet gels were 185, 148 and 140 nm for CF 1, CF 3 and CF 5, respectively. It may be assumed that these differences are responsible for the overall differences in physical properties between acid- and rennet-induced gels.

Acid gels obtained from skim milk (CF 1) showed a loose network with large pore size. As the milk concentration was increased the network of the gel became denser and thus the average pore size decreased from 1.28 μm in skim milk gel to 0.33 and 0.26 μm in acid gels obtained from CF 3 and CF 5, respectively. Additionally, fusion of casein micelles was observed in all acid gels made from CF 1, CF 3 and CF 5. The micelle size also decreased from 150 nm in the skim milk gel to an average diameter of 130 and 110 nm for CF 3 and CF 5 gels, respectively (Figure 5.4a,b). Differences in microstructure resulting from changes in milk composition by ultrafiltration may influence the physical properties of the gels and consequently the structure and texture of the cheese derived from UF gel. Since the basic structure of the protein network was already set at the gel forming stage, this structure was essentially not changed during the later stages of cheesemaking (Green et al., 1981).

Rennet gels obtained from skim milk and ultrafiltered skim milk retentates with 3- and 5-fold concentrations revealed slow changes in the gel microstructures as a function of time after rennet clotting. However, under the same conditions acid gels obtained from skim milk and retentates reached an end point structure very rapidly. Once the structure of acid gels was established, no further change occurred in the network structure as a function of time. The acid gel network structure was similar to that of the aged rennet gels, i.e. 60 min after rennet coagulation time.

As the protein concentration in retentates was increased, the network structure of both acid and rennet gels became denser while the pore and casein micelle sizes decreased. Furthermore, the rennet gels exhibited distinctive cracks throughout the network at the rennet clotting time. These cracks were much more pronounced in rennet gels derived from 5-fold retentates than those from 3-fold retentates.

However, when more time was allowed, at 30 min after rennet clotting time these cracks disappeared. Cracks were absent in acid gels at the same protein concentrations and also in rennet gels derived from skim milk. Therefore gel density, casein micelle size, and the cracks present in the gels - all reflecting the binding forces within the gels and the increased levels of the protein in skim milk retentates used in clotting - may influence the physical properties of the gels and consequently the structure and texture of the final cheese products.

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6. SUMMARY AND RECOMMENDATIONS

Active research and development concerning the UF process in cheese manufacturing have focused on the increase in cheese yield and UF cheese quality. Many attempts have been made to produce UF cheese with quality characteristics (texture and flavor) comparable to those of traditional cheeses. Soft cheeses with low total solids content which are normally consumed fresh and ripened soft cheese have been manufactured successfully by the application of UF process. In ripened soft cheeses, such as UF Feta or UF Blue cheese, the flavorful compounds released from lipolytic breakdown mask any flavor defects, such as the acid/bitter taste resulting from excessive minerals. However, in hard cheese varieties the situation is more complex. In these cheese types which have traditionally long ripening periods, the characteristic texture and flavor depend mainly upon proteolysis (Lawrence, 1989). Many factors are involved and play significant roles in the successful production of such cheese varieties from UF milk. Basic knowledge of physical, chemical, biological and structural properties of UF milk and factors involved in controlling these properties are fundamental requirements before its successful application can be achieved. Some of the most important properties and changes in milk as the result of UF were the subject of this study. Following is the summary of the findings obtained from the work undertaken:

6.1 Effect of UF on the Composition and Buffer Capacity of Skim Milk

Concentration of UF skim milk resulted in increased concentration of macromolecules: casein, whey proteins and fat in direct proportion to the concentration factor (CF). In contrast the concentration of minerals: Ca, P, Mg, K, and Na increased linearly but not in direct proportion to CF . The increase in casein, whey proteins, and minerals resulted in a linear increase in maximum buffer capacity of the UF milk retentate with increased CF. These relationships can be established and used in practice to determine the change in concentration of milk constituents and maximum buffer capacity at various CF when milk is subjected to UF process under the same conditions, such as pH, temperature and pressure, etc.

6.2 Buffer System Intensity Change in UF Milk Retentate

Casein, whey proteins and minerals make an important contribution to the buffer system of milk. From this study, it can be concluded that in skim milk, minerals contributed the highest proportion to the buffer system intensity (58.6%), whey proteins the lowest (5.4%) and casein intermediate (36.0%). When the milk was ultrafiltered to higher concentration, the proportion of their contributions to the buffer system intensity was changed. The relative contribution of minerals decreased whereas those of casein and whey proteins increased steadily with increasing CF. For example, in milk ultrafiltered from CF 2 to CF 5, casein contribution to the buffer system increased from 47.2% in CF 2 to 53.8% in CF 5 and became the highest while the contribution of minerals decreased from 46.7% in CF 2 to 36.6% in CF 5 and became intermediate and whey proteins changed from 6.1% in CF 2 to 9.7% in CF 5. However, there was an increase in buffer system intensity as the result of increased mineral concentration in CF 5 about 2 times greater than that of skim milk (Figure 1.9). Minerals influence not only buffer capacity but also basic structure and therefore texture, stretchability and meltability of UF cheese (Lawrence et al., 1983; Lawrence and Gilles, 1986). In practice mineral content can be controlled by adjusting pH during UF (Sutherland and Jameson, 1981) or coupled with diafiltration (Glover, 1985).

6.3 Technological Importance of Buffer Capacity in UF Milk Retentate

The relatively high buffer capacity of UF milk retentate in comparison to normal milk is a major factor that influences the characteristic texture and flavor of UF cheese. A high buffer capacity results in UF cheese with higher pH than in traditional cheese. This may lead to the unsatisfactory quality of UF hard cheese since the pH at whey draining is a critical point determining the mineral content, which in turn influences the basic structure and the retention of calf rennet and plasmin in the cheese curd. The final curd pH after salting influences enzymatic activities of rennet, plasmin and starter culture which are necessary for cheese maturation (Lawrence and Gilles, 1986; Thomas and Pearce, 1981). Therefore in cheese manufacture the pH change (or acid development) with time during cheesemaking is very important and it is used as a process control in the dairy plant. The high buffer capacity in UF milk causes a significant difference in the pH-time control chart from normal milk during cheesemaking.

Buffer capacity may also play a significant role in further processing and utilization of UF retentate as a raw material for other dairy and food products. Furthermore, the high buffer capacity also makes it more difficult to achieve the desired low pH to suppress growth of undesirable microorganisms which may cause spoilage and food poisoning (Kosikowski, 1986). The pH of UF retentate could be adjusted by direct acidification and the quantity of weak acid required to change pH by one unit was greater than that of strong acid and both were dependent upon the CF.

6.4 Effect of UF on Growth and Activity of Starter Cultures

A large inoculum size in proportion to the protein content of retentate was used for the normal activity test at 32°C in order to overcome the high buffer capacity effect. It was found that there was an uncoupling of growth from lactic acid production as indicated by the increase in doubling time for growth of all three strains studied while the doubling time for lactic acid production decreased with increasing CF. The reasons for the uncoupling of growth from lactic acid production are unknown. Both Mistry and Kosikowski (1985) and Turner and Thomas (1975) suggested that it may be due to the overcrowding of the starter bacterial cells as a result of the high inoculum size, and that this may influence the growth while acid production is unaffected. This is supported by the observed increase in specific lactic acid production with increasing inculum size in this study. Although large amounts of lactic acid were produced in more concentrated UF milk the changes in pH were less due to the higher buffer capacity. As a result, pH 4.6 was not achieved at CF 4 and CF 5 retentates even after incubation for 10 h at 32°C and yet the amount of lactic acid produced in CF 5 retentate was about three times greater than that required for the skim milk to achieve pH 4.6.

A simulated Cheddar cheese manufacturing test proposed by Okibo et al. (1985) for determining the activity and inoculum size of starter culture necessary to give the desired pH in normal milk was applied to the UF milk. The behavior of starter cultures under cheese manufacturing conditions differs from that observed for the normal activity test under laboratory conditions (Heap and Lawrence, 1981; Pearce, 1969). The results obtained in this study supported the concept that the conditions simulating cheese manufacturing are highly recommended for testing the activity of starter culture. The simulated cheese manufacturing test is as simple as the normal activity test but very effective in determining the inoculum size necessary to obtain the desired pH. The percent of inoculum can be obtained from the following relationship: $y = a + b \ln x$ where y is the percent inoculum size, x is the desired pH change, a is intercept and b is the slope. This test could be recommended at the dairy plant level to determine inoculum size for cheesemaking from UF milk.

From the simulated cheese manufacturing test it was found that it was possible to overcome the effect of buffer capacity to obtain the desired pH up to CF 4 by the use of a 5% inoculum of a strongly proteolytic strain. However, for retentate of CF 5 the use of 10% inoculum, which is impractical in cheese manufacture, failed to overcome the buffer effect. However, factors other than acid development must be considered in the selection of the starter strain. High levels of starter proteinase from high inoculum size could result in the formation of a high proportion of hydrophobic peptides, derived from casein, leading to bitterness in cheese (Law and Kolstad, 1983; Law and Sharpe, 1978). For cheesemaking from normal milk, the use of mixed protienase positive (Prt$^+$) and deficient (Prt$^-$) strains, with high proportion of Prt$^-$, or the use of a high concentration of a Prt$^-$ strain alone resulted in a product with less bitter flavor development (Mills and Thomas, 1980; Richardson et al., 1983). A similar approach is worth trying in order to overcome the high buffer capacity effect in highly concentrated UF milk with less bitter flavor development. Direct acidification of the milk before UF or coupled with diafiltration during UF (Glover, 1985) could successfully overcome the buffer capacity effect.

6.5 Effect of UF on Casein Micelle Size Distribution, Average Diameter and Volume Distribution of Casein Micelles

Transmission electron micrographs of casein micelles obtained from UF skim milk to 3- and 5-fold concentrates illustrated roughly spherical particles with a range of size similar to those seen in skim milk. However, there was a significant change in casein micelle size distribution when the milk was ultrafiltered to higher concentrations. The maximum proportion of casein micelles was changed from 80-100 nm in diameter in skim milk to 60-80 nm in 5-fold concentrate. At higher CF the number of casein micelles with diameter less than 80 nm increased whereas those with diameters greater than 100 nm decreased. Consequently, the volume distribution and average diameter of the casein micelles were altered. There was a highly significant decrease in casein micelle diameter from 118 nm in normal skim milk to 92 and 87 nm in CF 3 and CF 5 retentates, respectively ($P < 0.01$). The change in composition of casein and minerals particularly Ca and P as a result of UF may be responsible for the change in casein micelle size distribution, volume distribution and average diameter of casein micelles of the UF retentate and therefore influencing its processing and rheological properties.

6.6 Effect of UF on Microstructure of Rennet and Acid Gels

Although both acid and rennet coagulants cause destabilization and aggregation of casein micelles to form gel or curd, their actions to destabilize the casein micelles are by completely different mechanisms. Due to the slow drop in negative charge and slow removal of the "hairy layer" (which contributes to the electrostatic and steric stabilities of casein micelles) by rennet coagulation, rennet gel obtained from skim milk and its UF retentates exhibited slow changes in microstructure as a function of time elapsed after rennet clotting time (RCT). For UF milk retentate the structure of rennet gels showed a denser network, pore size decrease and an increase in micelle size that paralleled the time elapsed after RCT. However, all acid gels derived from the same UF retentates and condition of clotting reached the end point structure rapidly as a result of rapid neutralization of the negative charge and rapid reduction of the hairy layer of the casein micelles. In addition dissociation of the colloidal calcium phosphate (CCP) from the casein micelles occurs with acid coagulation. At pH 5.0, CCP is completely removed from the casein micelles (Heertje et al., 1985). So, with acid gels no further change in the gel structure occurred as a function of time once the end point structure had been reached (Knoop and Peters, 1975). The network structure of all acid gels obtained in this study were similar to those of aged rennet gels at 60 min after RCT. However, the gel obtained exibited a denser network structure, smaller pores and smaller casein micelle size than that of rennet gels of corresponding CF at 60 min after RCT. These differences in microstructure, CCP and the way the caseins are aggregated may be responsible for the overall difference in physical properties between acid and rennet gels.

As the milk concentration increased, the network structure of both acid and rennet gels became denser while pore size and micelle size decreased. This may be due to a decrease in mean free distance of casein micelles as the casein concentration increased. The mean free distance of casein micelles is reduced from three-micelle diameter present in normal milk to less than a one-diameter diameter in CF 4 milk retentate (Green et al., 1981). This may restrict movement of the casein micelles and restrict the choice of their position. Therefore it may affect the type of gel structure with stronger links in the casein matrix, and consequently, it may be responsible for the atypical body and texture (hard, crumbly, dry and granular) of hard cheese varieties manufactured from high UF milk retentate.

Furthermore, the rennet gels obtained from CF 3 and CF 5 retentate showed curved cracks or narrow void spaces throughout the gel network at RCT which were much more pronounced in gels derived from CF 5 retentate. These cracks were absent in rennet gels obtained from skim milk and in all acid gels derived from all retentates studied. However, when more time was allowed for 30 min after RCT more casein micelles were incorporated into the gel network. Therefore all the cracks disappeared in both CF 3 and CF 5 gel network structures at 30 min after RCT. These cracks may be a result of the incomplete incorporation of casein micelles into the gel network and could be responsible for a coarse network structure in the cheese obtained from high UF retentate. Green et al. (1981) observed the microstucture of the cheese at different stages during Cheddar cheese manufacturing: cutting, whey draining, pressing and ripening for 5 and 28 weeks. They concluded that the protein increased the coarseness in direct proportion to the CF of the milk. The relative difference in structure was maintained throughout cheesemaking into the mature cheese. Therefore the basic structure of the protein network was laid down during the curd formation and was not fundamentally changed later in cheese manufacturing. In normal milk about 90% of casein micelles are incorporated into the curd at RCT. As the milk is concentrated the amount of casein micelles incorporated into the curd at RCT decreased (Dalgleish, 1981). Those micellles which are free at RCT will later be incorporated into the gel in a different manner from those incorporated before RCT either with gel matrix or with other free micelles. The change in the mechanism of casein micelle aggregation may be responsible for the unsatisfactory texture of the cheese (Fox, 1984; Green et al., 1981).

The incomplete incorporation of casein micelles into the gel network may result in less developed structure of the UF curd which renders it more fragile and thus more susceptible to physical damage. Furthermore, there is less whey from the UF curd to cushion the curd from physical damage during manufacturing (Van Leeuwen et al., 1984). Consequently, UF curd is very difficult to handle, cut, heat, stir and transport by conventional procedures. Therefore it is necessary to develop novel equipment for the manufacture of UF cheese (Coton, 1986; Sutherland and Jameson, 1981). At present, research is being done to investigate how best to handle UF curd for cheesemaking (Lawrence, 1989).

In conclusion UF caused significant changes in milk composition, physical properties, casein micelle size distribution and gel structure. These findings are crucial and will provide more information for the practical utilization of UF milk as a raw material for dairy and food products.

6.7 Recommendations for Future Work

The use of UF process for the manufacture of hard cheese varieties is not completely satisfactory. At present the successful manufacture of the UF cheese is still a matter of trial and error and complete scientific explanations are not yet available (Lawrence, 1989). More research is needed especially in the following areas:

i. The partitioning between mineral contents, particularly Ca in soluble and colloidal forms in UF retentate during the cheese manufacturing. Ca influences the basic structure of UF cheese and stretchabilty and meltability of Mozarella type cheeses. It is an important parameter that must be controlled. At present, it is much more difficult to control Ca content in UF cheesemaking than in traditional cheesemaking.

ii. The proteolytic activities of rennet, plasmin and bacterial starter on UF cheese during ripening. The proteolytic breakdown of casein is a key factor in the ripening of hard cheeses.

iii. The involvement of whey proteins in UF cheese quality. The breakdown of whey proteins during cheese ripening influences subsequent texture and flavor development of the UF cheese.

As more research results are obtained the use of UF processing will become more widespread. Soft cheese varieties (Camembert, Feta and Blue cheese, etc.) have already been successfully manufactured from UF milk because of their economical, technical and nutritional advantages. The use of UF process to standardize the protein content (variation of protein due to seasonal change) in UF milk is a common dairy practice in Europe in order to obtain greater uniformity of cheese composition and thus, cheese quality. The use of low concentrate (LCR), UF milk concentrated up to 2-fold concentration allows the existing conventional methods and equipment to be used with marked increase in vat and space capacities.

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